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TALL OIL

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prepared for
THE FOOD AND DRUG ADMINISTRATION
DEPARTMENT OF HEALTH, EDUCATION AND WELFARE

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Hormones from Swedish Sulfate Pulp

Chem. Eng. News 25(7): 454 (1947)

One of the by-products obtained in the manufacture of sulfate pulp is the so-called phytosterol group which serves as a basic ingredient for the manufacture of many remedial agents, including hormones. The Swedish Pulp Company has recently begun manufacture of phytosterol from Swedish pine, and its finished product seems capable in many cases of replacing cholesterol, at present difficult to obtain from its usual source, the spinal cord of calves. Methods for extracting the phytosterol groups in a pure state and on a large scale have been developed by the company's chemists and engineers over a number of years. Output is expected to cover the world's needs for some time.

Chem. Eng. News, 45(7): 16-17, 19, 1967

Tall oil rosin output climbs 10% yearly

Abundant raw material and low price account for surge in activity of sulfate naval stores

That least glamorous of chemical raw materials—naval stores—calls for some attention this year and next. That part of it called sulfate naval stores (or more accurately tall oil) will be getting a lot more capacity. Three companies are adding a total of 210,000 tons a year of tall oil fractionation capacity to bring the industry's total to more than 875,000 tons by June 1968. The new capacity represents an expansion of about 32%.

The additional capacity is coming from Hercules, Arizona Chemical, and Crosby Chemicals. Hercules (biggest U.S. company in naval stores in general and tall oil in particular) is building a plant at Hattiesburg, Miss. The 90,000 ton-a-year unit will be completed at the end of this year. It will be the company's fourth tall oil fractionation plant in the U.S.

Arizona Chemical (jointly owned by American Cyanamid and International Paper) is adding 40,000 tons capacity to its existing 80,000 ton-a-year unit at Panama City, Fla. Crosby Chemicals is adding 30,000 tons to its 45,000 ton-a-year plant at Picayune, Miss., and is building a 50,000 ton-a-year tall oil fractionation unit at De Ridder, La. The De Ridder plant will be completed by June 1968, the Picayune expansion this fall.

Why all this tall oil fractionation capacity? The answer lies in a combination of raw material availability, properties, and price.

Naval stores rosin consists primarily of tricyclic monocarboxylic acids (molecular formula $C_{20}H_{30}O_2$). The main acids in tall oil rosin are abietic, dehydroabietic, neoabietic, dihydroabietic, palustric, pimaric, and isoprimaric acids.

Originally naval stores were known as the pitch and rosin recovered from pine wood to caulk wooden ships and tar their rigging. There are three distinct types of naval stores: gum, steam-distilled wood, and sulfate naval stores.

Gum naval stores (or oleoresin) are obtained by tapping living pine trees. The process entails removing a new strip of bark from each tree about every two weeks, spraying the resulting wound with sulfuric acid to activate the flow of gum exudate, and collecting the gum in cups attached to the trees. The gum is sent to processing plants where turpentine from the gum is removed by steam distillation, and gum rosin obtained as residue.

Wood naval stores involves producing rosin from virgin stumps, primarily of longleaf pine. When stumps of such trees are left in the ground for not less than 25 years after the tree is cut, the stumps are rich in rosin. They are removed with earth-moving equipment and trucked or shipped by rail to plants where they are cleaned, shredded, and extracted with solvent. The extract is distilled to produce wood rosin, pine oil, and wood turpentine.

The third type of naval stores is sulfate naval stores or tall oil. Crude tall oil is a by-product of kraft pulp mills removed in the form of soap skimmings (called black liquor skimmings) which are acidified to tall oil rosin and fatty acids.

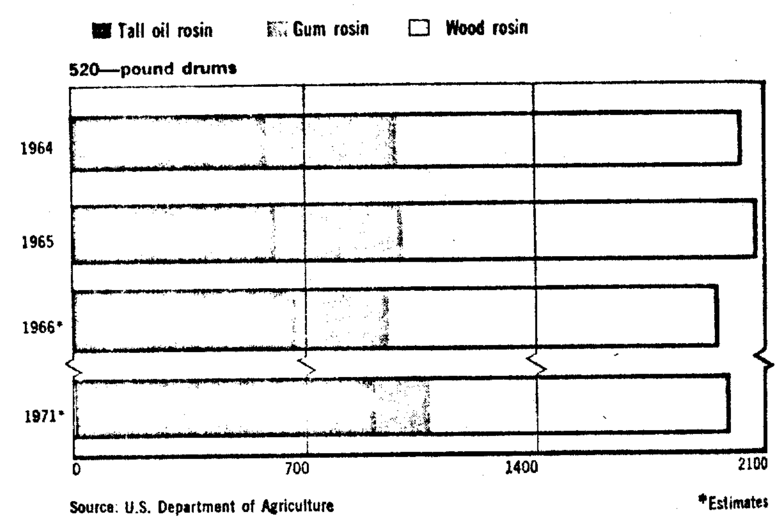
The fatty acids portion (1 ton of crude tall oil yields about 500 pounds of fatty acids, 800 pounds of rosin acids, and 700 pounds of secondary products comprising distilled tall oil, tall oil heads, and tall oil pitch) totaled about 337 million pounds in 1966. These acids find a ready market in many applications such as protective coatings, soaps, detergents, disinfectants, intermediate chemicals, flotation, and tallate driers. Indeed, so successful is the market for tall oil fatty acids that inventory last year averaged 15 million pounds (about 4.5% of production), one of the lowest in any industry, according to Douglas E. Campbell, secretary, tall oil products division, Pulp Chemicals Association.

What makes tall oil rosin particularly attractive as a raw material is its availability. The gum rosin industry, on the other hand, scars trunks of living pine and involves manual tasks that are costly and time-consuming. And the wood rosin industry depends on the availability of aged tree stumps whose removal and transportation are becoming increasingly costly. But crude tall oil is a "waste" product of pulp making. In fact, if the black liquor skimmings did not yield some useful products, they could be a disposal problem for pulp mills.

The growth of new capacity in kraft pulp mills has made available a large quantity of crude tall oil. Future growth of the paper industry—about 5% a year—will provide increasing supplies of raw material for tall oil fractionators. The latter are also continuously improving their methods of separating rosin (and fatty acids) from other constituents of tall oil to provide better quality products at lower cost.

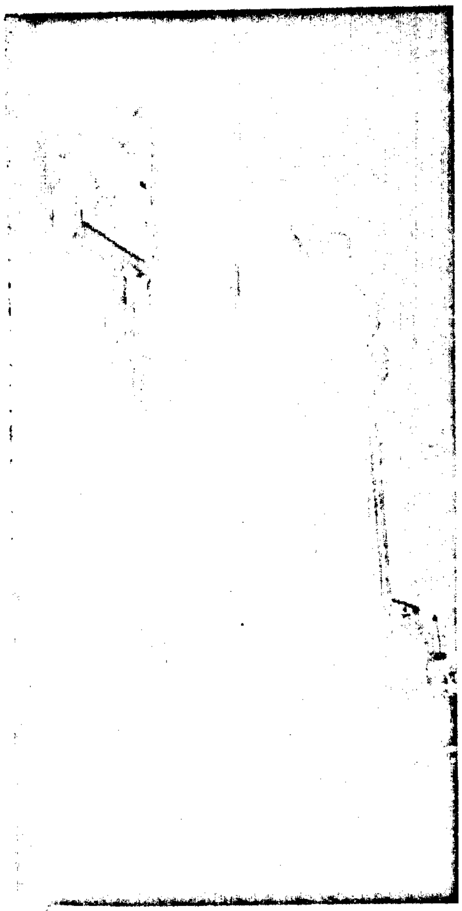
Although total U.S. rosin production has been changing only slightly

Tall oil rosin output will equal that of wood rosin by 1971



Source: U.S. Department of Agriculture

*Estimates



FRACTIONATING COLUMN. At Panama City, Fla., Arizona Chemical is adding 40,000 tons a year of tall oil capacity to meet the demand for rosin

the past few years, the relative shares of each type of rosin have been changing significantly. In the 1966-67 crop year, for example, the supply of rosin was comprised of wood, 51.4%; tall oil, 34.7%; and gum, 13.9%. In 1961, the shares were wood, 53.9%; tall oil, 23.0%; and gum, 23.1%. In these five years, the shares of both steam-distilled wood and gum rosin have declined in the face of the continuing growth of output of tall oil rosin.

Notwithstanding support from the Forest Service's naval stores conservation program, the gum naval stores industry (80% of which is concentrated in Georgia) has suffered a very sharp decline.

For steam-distilled wood, the declining supply of virgin pine stumps and increasing costs of collecting the stumps are basic factors accounting for the decline in wood rosin output. The U.S. Department of Agriculture estimates that, as both gum and wood rosin output falls, by 1971 tall oil rosin output will be moving close to 50% of all rosin output in the U.S. Furthermore, output of tall oil rosin by then will be nearly 100% more than it was in 1961—a substantial growth for a 10-year period.

The end-use pattern for rosins is also changing as tall oil rosin makes inroads into rosin consuming industries. Paper size accounts for the largest single use of all rosin, about 38%. Gum rosin was initially used for paper size since it was the only one suitable. When wood rosin became suitable as a result of research, it began to replace gum and eventually became the most important rosin for paper size. But tall oil has outpaced both gum and wood rosin to pick up some 60% of the total rosin used in size manufacture. Wood rosin's share is 30% and gum's 10%.

Synthetic rubber is the second most important outlet for rosin and consumes about 13% of all rosin output. In making styrene-butadiene rubber, disproportionated rosin soaps, fatty acid soaps, and combinations of both are used as emulsifiers in polymerization. In 1963, Arizona Chemical came up with the first commercial tall oil disproportionated rosin soaps to challenge wood rosin as the only source of rosin for disproportionation. (Disproportionation decreases the number of double bonds in the abietic acid in tall oil rosin and makes for a more stable material.) Today, producers of SBR are getting lower cost emulsifier reagents mainly because of the successful disproportionation of tall oil rosin.

Actually, expansion of the use of disproportionated tall oil rosin in the elastomer industry could be affected by changing technology. One such change is that newer elastomer types, such as polybutadiene, polyisoprene, and the ethylene-propylene diene monomers are made by solvent polymerization and do not require emulsifiers. Yet, the tall oil industry feels confident. As Hercules vice president Don Sheffield points out, there should be a growing need in the rubber industry for tall oil derivatives (including the fatty acids) for rubber compounding and other rubber formulations.

The adhesives industry is the third most important market for rosin. Large quantities of rosins, modified rosins, and rosin derivatives are used to make various types of adhesives. In the most recent crop year, probably more than 90 million pounds of rosin were used in adhesives. By 1970, rosin demand for use in adhesives may be near 135 million pounds.

The most important adhesive applications for rosins include the pressure-sensitive, the hot melt, and the elastomer-based latex and solvent rubber cements. According to Joe Garbarino, manager, commercial development, of Arizona Chemical, these adhesive markets are experiencing excellent growth which will continue.

In pressure-sensitive adhesives, rosin derivatives are successfully competing with a host of other resins including polyterpenes and petroleum and coal tar hydrocarbon resins. Mr. Garbarino says that the development of new derivatives of tall oil rosin products will assure greater participation of tall oil in these markets.

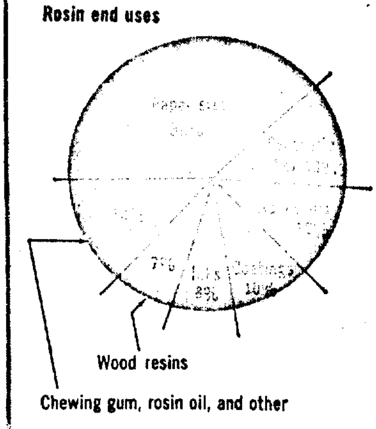
The hot melt products offer considerable promise for further growth in tall oil rosin per se, and at the expense of gum and wood rosins in both hot-melt coatings and adhesives. Hercules' Don Sheffield agrees with this prediction of Mr. Garbarino's. Hot melts are those based on combinations of low-molecular-weight waxes, such as paraffin wax, and resins which have high strength and molecular weight, such as polyvinyl acetate, and copolymers of ethylene and vinyl acetate.

There are a number of new, improved products under development that would push further growth of tall oil rosin derivatives in the rapidly expanding adhesives industry. Properties of these products show them to be equal or superior to other higher priced resins currently in use. Arizona Chemical sees such growth in hot melts alone that the materials will probably consume more than 50 million pounds of rosin by 1970.

Protective coatings account for about 11% of total rosin consumption in the U.S., and they are going to continue to be an important outlet for tall oil rosin in the future, according to

Paper, rubber, adhesives are big rosin users

Rosin end uses



Source: C&EN Estimates

Hercules has biggest tall oil fractionation capacity

COMPANY	LOCATION	CAPACITY*	REMARKS
Arizona Chemical	Panama City, Fla.	80	Adding 40,000 tons by June 1967
	Springhill, La.	40	
Crosby Chemicals	Picayune, Miss.	45	Adding 30,000 tons by fall 1967
	De Ridder, La.		New 50,000 ton-a-year plant will be completed by June 1968
Glidden	Port St. Joe, Fla.	50	
Hercules	Franklin, Va.	65	
	Portland, Ore.	25	
	Savannah, Ga.	65	
	Hattiesburg, Miss.		New 90,000 ton-a-year plant will be completed by end of 1967
Monsanto-Emery	Nitro, W.Va.	40	
Owens-Illinois	Valdosta, Ga.	10	
Tenneco	Bay Minette, Ala.	36	
	Oakdale, La.	29	
Union-Camp	Savannah, Ga.	110	
West Virginia Pulp & Paper	Charleston, S.C.	50	
Ziegler	Chicago, Ill.	20	
Total current capacity		665	
Total capacity when expansions and new plants are completed		875	

* In thousands of tons of crude input per year.

Mr. Sheffield. Rosins, modified rosins, and rosin derivatives are still widely used to make various protective coatings such as varnishes and alkyds. Although total rosin consumption for coatings has been declining in recent years because new technology has increased the use of nonrosin-containing synthetic resins, there remains a bright picture for tall oil rosin as improvement in the quality of tall oil rosin is realized.

In printing inks all three kinds of rosins are used to the tune of about 7.5% of total rosin consumption. Here, though, the rosins most used have been wood and gum; currently gum rosin is the more important. But tall oil producers expect to gain in this market in the near future.

End uses for tall oil rosin and derivatives are many—from adhesives and asphalt additives through linoleum and varnishes. What is important about the future of tall oil is a combination of properties that indicate its potential for growth. The change-over to biodegradable detergents, for instance, has given new emphasis to tall oil since tall oil rosin and disproportionated tall oil rosin soaps are biodegradable.

Another important aspect of rosins and tall oil rosin is the approval by the Food and Drug Administration for their use in food packaging applications. Chemical reactivity of tall oil rosin (principally involving the carboxyl group and double bonds) means it can be used to produce salts, soaps, esters, amines, amides, nitriles, and Diels-Alder adducts. The double bonds allow isomerization, disproportionation, hydrogenation, dimerization, and polymerization.

There remain some technical problems for tall oil, however. Mr. Sheffield points to sulfur content as one of them. Current refining methods don't eliminate sulfur in tall oil and its derivatives. This bars their use in some applications. For instance, it is difficult to make hydrogenated rosin because the sulfur poisons catalytic systems.

But technical problems aside, there remains one most important factor that will give tall oil rosin a distinct advantage in the overall rosin picture—price. Tall oil rosin is currently priced at 9.8 cents a pound (carload drums, f.o.b. plant). Gum rosin is priced at 10.9 cents a pound, and wood rosin 12.0 cents for equivalent color grades. With gum rosin output dropping nearly 27% in the current crop year, and wood rosin facing a slow but certain decline, tall oil rosin's price combined with its end-use versatility will give it an increasingly powerful hold in the total rosin market.

Title 21—FOOD AND DRUGS

Chapter I—Food and Drug Administration, Department of Health, Education, and Welfare

SUBCHAPTER B—FOOD AND FOOD PRODUCTS

PART 121—FOOD ADDITIVES

Subpart D—Food Additives Permitted in Food for Human Consumption

GLYCEROL ESTER OF TALL OIL ROSIN

The Commissioner of Food and Drugs, having evaluated the data in a petition (FAP 9A2402) filed by American Cyanamid Co., Wayne, N.J. 07470, and other relevant material, concludes that the food additive regulations should be amended to provide for safe use of glycerol ester of tall oil rosin as a plasticizing material (softener) in chewing gum base. Therefore, pursuant to provisions of the Federal Food, Drug, and Cosmetic Act (sec. 409(c)(1), 72 Stat. 1786; 21 U.S.C. 348(c)(1)) and under authority delegated to the Commissioner (21 CFR 2.120), § 121.1059 is amended in paragraph (a) by alphabetically inserting in the list of substances under "Plasticizing Materials (Softeners)" a new item as follows:

§ 121.1059 Chewing gum base.

* * * * *

(a) * * *

MASTICATORY SUBSTANCES

* * * * *

PLASTICIZING MATERIALS (SOFTENERS)

* * *	* * *
Glycerol ester of tall oil rosin.	Having an acid number of 5-12, a softening point (ring and ball) of 80°-88° C., and a color of N or paler. The ester is purified by steam stripping.
* * *	* * *

* * * * *

Any person who will be adversely affected by the foregoing order may at any time within 30 days from the date of its publication in the FEDERAL REGISTER file with the Hearing Clerk, Department of Health, Education, and Welfare, Room 5440, 330 Independence Avenue SW., Washington, D.C. 20201, written objections thereto, preferably in quintuplicate. Objections shall show wherein the person filing will be adversely affected by the order and specify with particularity the provisions of the order deemed objectionable and the grounds for the objections. If a hearing is requested, the objections must state the issues for the hearing. A hearing will be granted if the objections are supported by grounds legally sufficient to justify the relief sought. Objections may be accompanied by a memorandum or brief in support thereof.

Effective date. This order shall become effective on the date of its publication in the FEDERAL REGISTER.

(Sec. 409(c)(1), 72 Stat. 1786; 21 U.S.C. 348(c)(1))

Dated: January 6, 1970.

R. E. DUGGAN,
Acting Associate Commissioner for Compliance.

[F.R. Doc. 70-390; Filed, Jan. 13, 1970; 8:46 a.m.]

and the regulations in this part for the promulgation of a regulation.

(b) "Reasonable grounds" shall include an explanation showing wherein the person has a substantial interest in such regulation and an assertion of facts (supported by data if available) showing that new information exists with respect to the food additive or that new uses have been developed or old uses abandoned, that new data are available as to toxicity of the chemical, or that experience with the existing regulation or exemption may justify its amendment or repeal. New data should be furnished in the form specified in § 121.51 for submitting petitions.

§ 121.75 Exemption for investigational use and procedure for obtaining authorization to market edible products from experimental animals.

A food additive, including one that is a new drug or antibiotic, or foods containing such a food additive, intended for investigational use by qualified experts shall be exempt from the requirements of section 409 of the act under the following conditions:

(a) If intended for investigational use in vitro or with laboratory research animals, it bears a label which states prominently, in addition to other information required by the act:

Caution—Contains a new food additive (or new drug, or antibiotic) for investigational use only in laboratory research animals, or for tests in vitro. Not for use in humans.

(b) If intended for clinical investigational use in animals other than laboratory research animals, and the edible products of the animals are to be marketed as food, permission for the marketing of the edible products as food has been requested by the sponsor, and authorization has been granted by the Food and Drug Administration in accordance with §130.3a of this chapter, or by the Department of Agriculture in accordance with § 309.20 of Title 9, Code of Federal Regulations, and it bears a label which states prominently, in addition to other information required by the act, the following:

Caution—Contains a new food additive (or new drug, or antibiotic) for use only in investigational animals in clinical trials. Not for use in humans.

Edible products of investigational animals are not to be used for food unless authorization has been granted by the U.S. Food and Drug Administration or by the U.S. Department of Agriculture.

[31 F.R. 4891, Mar. 24, 1966]

Subpart B—Exemption of Certain Food Additives From the Requirement of Tolerances

§ 121.101 Substances that are generally recognized as safe.

(a) It is impracticable to list all substances that are generally recognized as safe for their intended use. However, by way of illustration, the Commissioner regards such common food ingredients as salt, pepper, sugar, vinegar, baking powder, and monosodium glutamate as safe for their intended use. The lists in paragraph (d) of this section include additional substances that, when used for the purposes indicated, in accordance with good manufacturing practice, are regarded by the Commissioner as generally recognized as safe for such uses.

(b) For the purposes of this section, good manufacturing practice shall be defined to include the following restrictions:

(1) The quantity of a substance added to food does not exceed the amount reasonably required to accomplish its intended physical, nutritional, or other technical effect in food; and

(2) The quantity of a substance that becomes a component of food as a result of its use in the manufacturing, processing, or packaging of food, and which is not intended to accomplish any physical or other technical effect in the food itself, shall be reduced to the extent reasonably possible.

(3) The substance is of appropriate food grade and is prepared and handled as a food ingredient. Upon request the Commissioner will offer an opinion, based on specifications and intended use, as to whether or not a particular grade or lot of the substance is of suitable purity for use in food and would generally be regarded as safe for the purpose intended, by experts qualified to evaluate its safety.

(c) The inclusion of substances in the list of nutrients does not constitute a finding on the part of the Department that the substance is useful as a supplement to the diet for humans.

Glycerin.
Guar gum.
Invert sugar.
Iron, reduced.
Locust bean gum (carob bean gum).
Magnesium carbonate.
Magnesium chloride.
Magnesium hydroxide.
Magnesium sulfate.
Methyl and ethyl acrylate.
Mono- and diglycerides from glycerolysis of edible fats and oils.
Oleic acid.
Oxides of iron.
Potassium sorbate.
Propionic acid.
Propylene glycol.
Silicon dioxides.
Pulps from wood, straw, bagasse, or other natural sources.
Soap (sodium oleate, sodium palmitate).
Sodium aluminate.
Sodium carbonate.
Sodium chloride.
Sodium hexametaphosphate.
Sodium hydrosulfite.
Sodium hydroxide.
Sodium phosphoaluminate.
Sodium silicate.
Sodium sorbate.
Sodium sulfate.
Sodium thiosulfate (additive in salt).
Sodium tripolyphosphate.
Sorbitol.
Soy protein, isolated.
Sulfamic acid.
Sulfuric acid.
Starch, acid modified.
Starch, pregelatinized.
Starch, unmodified.
Sucrose.
Talc.
Urea.
Vanillin.
Zinc hydrosulfite.
Zinc sulfate.

(i) Substances migrating to food from cotton and cotton fabrics used in dry food packaging that are generally recognized as safe for their intended use, within the meaning of section 409 of the act, are as follows:

Acacia (gum arabic).
Acetic acid.
Beef tallow.
Calcium chloride.
Carboxymethylcellulose.
Coconut oil, refined.
Corn dextrin.
Cornstarch.
Fish oil (hydrogenated).
Gelatin.
Guar gum.
Hydrogen peroxide.
Japan wax.
Lard.
Lard oil.
Lecithin (vegetable).
Locust bean gum (carob bean gum).
Oleic acid.
Peanut oil.
Potato starch.
Sodium acetate.
Sodium bicarbonate.
Sodium carbonate.
Sodium chloride.
Sodium hydroxide.
Sodium sulfate.
Sodium silicate.
Sodium tripolyphosphate.
Sorbose.
Soybean oil (hydrogenated).
Stearic acid.
Talc.
Tall oil.
Tallow (hydrogenated).
Tallow flakes.
Tapioca starch.
Tartaric acid.
Tetrasodium pyrophosphate.
Urea.
Wheat starch.
Zinc chloride.

(Secs. 201(s), 409, 701(a), 52 Stat. 1055, 72 Stat. 1784, 1785 et seq., as amended; 21 U.S.C. 321(s), 348, 371(a)) [30 F.R. 15345, Dec. 23, 1965, as amended at 33 F.R. 5619, Apr. 11 1968; 34 F.R. 17064, Oct. 21, 1969; 35 F.R. 1049, Jan. 27, 1970]

§ 121.102 Adjuvants for pesticide chemicals.

Adjuvants, identified and used in accordance with § 120.1001 (c) and (d) of this chapter, which are added to pesticide use dilutions by a grower or applicator prior to application to the raw agricultural commodity, are exempt from the requirement of tolerances under section 409 of the act.

(Sec. 409, 72 Stat. 1785; 21 U.S.C. 348)

Subpart C—Food Additives Permitted in Feed and Drinking Water of Animals or for the Treatment of Food-Producing Animals

AUTHORITY: The provisions of this Subpart C issued under sec. 409, 72 Stat. 1785; 21 U.S.C. 348, unless otherwise noted.

§ 121.200 Definitions and interpretations applicable to Subpart C.

(a) Regulations prescribing conditions under which additives may be safely used in animal feed, animal feed supplements, concentrates, or premixes or in animals intended for food use shall not be construed to relieve such additives from the provisions of sections 505 and

. (butylcarbamoyl)-2-benzimidazole-...rbamate) and its metabolites contain...the benzimidazole moiety (calculated ...benomyl) in dried apple pomace when ...resent therein as a result of application ...preharvest and/or postharvest) of the ...ngicide to the raw agricultural com...modity apples.

[...F.R. 26007, Dec. 5, 1972]

§ 121.344 *O,O-Dimethyl* 2,2,2-trichloro-1-hydroxyethyl phosphonate.

A tolerance of 2.5 parts per million is ...tablished for residues of the insecticide O,O-dimethyl 2,2,2-trichloro-1-hydroxy...thyl phosphonate in dried citrus pulp ...hen present therein as a result of ap...plication of the insecticide to growing ...trus fruit.

[...FR 2681, Jan. 29, 1973. Redesignated at ... FR 6394, Mar. 9, 1973]

Subpart D—Food Additives Permitted in Food for Human Consumption

AUTHORITY: The provisions of this Sub...part D issued under sec. 409, 72 Stat. 1785; ... U.S.C. 348.

§ 121.1000 General provisions applicable to this subpart.

(a) Regulations prescribing conditions ...der which food additive substances ...may be safely used predicate usage under ...nditions of good manufacturing prac...ce. For the purposes of this subpart, ...d manufacturing practice shall be de...ed to include the following restric...ns.

...) The quantity of the substance ...ded to food does not exceed the amount ...asonably required to accomplish its in...nded physical, nutritive, or other tech...al effect in food.

(2) Any substance intended for use in ...n food is of appropriate food grade ...d is prepared and handled as a food ...redient.

(b) The existence of a regulation pre...bing safe conditions of use for a food ...tive shall not be construed to relieve ... use of the substance from compli...e with any other provision of the ...

(c) The existence of any regulation ...cribing safe conditions of use for a ...ent substance does not constitute a ...g that the substance is useful or ...red as a supplement to the diet of ...mans.

§ 121.1001 Ethoxyquin.

(a) Ethoxyquin (1,2-dihydro-6-eth...y-2,2,4-trimethylquinoline) may be safely used as an antioxidant for preservation of color in the production of chili powder, paprika, and ground chili at levels not in excess of 100 parts per million.

(b) In order to provide for the safe use of the additive in feed prepared in accordance with §§ 121.201 and 121.202, tolerances are established for residues of ethoxyquin in or on edible products of animals as follows:

5 parts per million in or on the uncooked fat of meat from animals except poultry.

3 parts per million in or on the uncooked liver and fat of poultry.

0.5 part per million in or on the uncooked muscle meat of animals.

0.5 part per million in poultry eggs.

Zero in milk.

§ 121.1004 Glyceryl-lacto esters of fatty acids.

Glyceryl-lacto esters of fatty acids (the lactic acid esters of mono- and diglycerides) may be safely used in food in accordance with the following prescribed conditions:

(a) They are manufactured from glycerin, lactic acid, and fatty acids conforming with § 121.1070 and/or oleic acid derived from tall oil fatty acids conforming with § 121.1237 and/or edible fats and oils.

(b) They are used in amounts not in excess of those reasonably required to accomplish their intended physical or technical effect as emulsifiers and plasticizers in food.

[30 F.R. 15845, Dec. 23, 1965, as amended at 36 F.R. 9628, May 27, 1971]

§ 121.1006 Maleic hydrazide.

A food additive known as maleic hydrazide (1,2-dihydro-3,6-pyridazinedione) may be present in potato chips when used in accordance with the following conditions:

(a) The food additive is present as a result of the application of a pesticide formulation containing maleic hydrazide to the growing potato plant in accordance with directions registered by the United States Department of Agriculture.

(b) The label of the pesticide formulation containing the food additive conforms to labeling registered by the United States Department of Agriculture.

(c) The food additive is present in an amount not to exceed 160 parts per million by weight of the finished food.

only as prescribed by subparagraph (1) of this paragraph.

[32 F.R. 9159, June 28, 1967, as amended at 34 F.R. 18421, Nov. 19, 1969]

§ 121.1047 Calcium stearoyl-2-lactylate.

The food additive calcium stearoyl-2-lactylate may be safely used in or on food in accordance with the following prescribed conditions:

(a) The additive, which is a mixture of calcium salts of stearoyl lactylic acids and minor proportions of other calcium salts of related acids, is manufactured by the reaction of stearic acid and lactic acid and conversion to the calcium salts.

(b) The additive meets the following specifications:

Acid number, 50–86.
Calcium content, 4.2–5.2 percent.
Lactic acid content, 32–38 percent.
Ester number, 125–164.

(c) It is used or intended for use as follows:

(1) As a dough conditioner in yeast-leavened bakery products and prepared mixes for yeast-leavened bakery products in an amount not to exceed 0.5 part for each 100 parts by weight of flour used.

(2) As a whipping agent in:

(i) Liquid and frozen egg white at a level not to exceed 0.05 percent.

(ii) Dried egg white at a level not to exceed 0.5 percent.

(iii) Whipped vegetable oil topping at a level not to exceed 0.3 percent of the weight of the finished whipped vegetable oil topping.

(3) As a conditioning agent in dehydrated potatoes in an amount not to exceed 0.5 percent by weight thereof.

(d) To assure safe use of the additive:

(1) The label and labeling of the food additive and any intermediate premix prepared therefrom shall bear, in addition to the other information required by the act, the following:

(i) The name of the additive.

(ii) A statement of the concentration or strength of the additive in any intermediate premixes.

(2) The label or labeling of the food additive shall also bear adequate directions of use to provide a finished food that complies with the limitations prescribed in paragraph (c) of this section.

[30 F.R. 15845, Dec. 23, 1965, as amended at 31 F.R. 10264, July 29, 1966, 33 F.R. 8594, May 12, 1968; 33 F.R. 15653, Oct. 23, 1968]

§ 121.1048 Lactylic esters of fatty acids.

Lactylic esters of fatty acids may be safely used in food in accordance with the following prescribed conditions:

(a) They are prepared from lactic acid and fatty acids meeting the requirements of § 121.1070(b) and/or oleic acid derived from tall oil fatty acids meeting the requirements of § 121.1237.

(b) They are used as emulsifiers, plasticizers, or surface-active agents in the following foods, when standards of identity do not preclude their use:

Foods	*Limitations*
Bakery mixes	
Baked products	
Cake icings, fillings, and toppings.	
Dehydrated fruits and vegetables.	
Dehydrated fruit and vegetable juices.	
Frozen desserts	
Liquid shortening	For household use.
Pancake mixes	
Precooked instant rice	
Pudding mixes	
Solid-state edible vegetable fat-water emulsions.	As substitutes for milk or cream in beverage coffee.

(c) They are used in an amount not greater than required to produce the intended physical or technical effect, and they may be used with shortening and edible fats and oils when such are required in the foods identified in paragraph (b) of this section.

[30 F.R. 15846, Dec. 23, 1965, as amended at 36 F.R. 9628, May 27, 1971]

§ 121.1050 Coumarone-indene resin.

The food additive coumarone-indene resin may be safely used on grapefruit, lemons, limes, oranges, tangelos, and tangerines in accordance with the following prescribed conditions:

(a) The food additive is manufactured by the polymerization of a crude, heavy coal-tar solvent naphtha meeting the following specifications:

(1) It is a mixture of indene, indan (hydrindene), substituted benzenes, and related compounds.

(2) It contains no more than 0.25 percent tar bases.

(3) 95 percent distills in the range 167° C.–184° C.

(b) The food additive meets the following specifications:

(b) The food additive is used or intended for use in the amount necessary for an emulsifier, stabilizer, or thickener in foods, except for those standardized foods that do not provide for such use.

(c) To assure safe use of the additive, the label and labeling of the additive shall bear the name of the salt of furcelleran that dominates the mixture by reason of the modification, e.g. "sodium furcelleran," "potassium furcelleran," etc.

§ 121.1070 Fatty acids.

The food additive fatty acids may be safely used in food and in the manufacture of food components in accordance with the following prescribed conditions:

(a) The food additive consists of one or any mixture of the following straight-chain monobasic carboxylic acids and their associated fatty acids manufactured from fats and oils derived from edible sources: Capric acid, caprylic acid, lauric acid, myristic acid, oleic acid, palmitic acid, and stearic acid.

(b) The food additive meets the following specifications:

(1) Unsaponifiable matter does not exceed 2 percent.

(2) It is free of chick-edema factor:

(i) As evidenced during the bioassay method for determining the chick-edema factor as prescribed in paragraph (c)(2) of this section; or

(ii) As evidenced by the absence of chromatographic peaks with a retention time relative to aldrin (RA) between 10 and 25, using the gas chromatographic-electron capture method prescribed in paragraph (c)(3) of this section. If chromatographic peaks are found with RA values between 10 and 25, the food additive shall meet the requirements of the bioassay method prescribed in paragraph (c)(2) of this section for determining chick-edema factor.

(c) For the purposes of this section:

(1) Unsaponifiable matter shall be determined by the method described in the most recent edition of "Official Methods of Analysis of the Association of Official Agricultural Chemists."

(2) Chick-edema factor shall be determined by the bioassay method described in Official Methods of Analysis of the Association of Official Agricultural Chemists, 10th Edition (1965), sections 26.087 through 26.091.

(3) The gas chromatographic-electron capture method for testing fatty acids for chick-edema shall be the method described in the "Journal of the Association of Official Analytical Chemists," Volume 50 (No. 1), pages 216–218 (1967), or the modified method using a sulfuric acid clean-up procedure, as described in the "Journal of the Association of Official Analytical Chemists," Volume 51 (No. 2), pages 489–490 (1968).

(d) It is used or intended for use as follows:

(1) In foods as a lubricant, binder, and as a defoaming agent in accordance with good manufacturing practice.

(2) As a component in the manufacture of other food-grade additives.

(e) To assure safe use of the additive, the label and labeling of the additive and any premix thereof shall bear, in addition to the other information required by the act, the following:

(1) The common or usual name of the acid or acids contained therein.

(2) The words "food grade," in juxtaposition with and equally as prominent as the name of the acid.

[30 F.R. 15845, Dec. 23, 1965, as amended at 31 F.R. 11215, Aug. 25, 1966; 32 F.R. 11432, Aug. 8, 1967; 33 F.R. 9016, June 19, 1968]

§ 121.1071 Salts of fatty acids.

The food additive salts of fatty acids may be safely used in food and in the manufacture of food components in accordance with the following prescribed conditions:

(a) The additive consists of one or any mixture of two or more of the aluminum, calcium, magnesium, potassium, and sodium salts of the fatty acids conforming with § 121.1070 and/or oleic acid derived from tall oil fatty acids conforming with § 121.1237.

(b) The food additive is used or intended for use as a binder, emulsifier, and anticaking agent in food in accordance with good manufacturing practice.

(c) To assure safe use of the additive, the label and labeling of the additive and any premix thereof shall bear, in addition to the other information required by the act, the following:

(1) The common or usual name of the fatty acid salt or salts contained therein.

(2) The words "food grade," in juxtaposition with and equally as prominent as the name of the salt.

[30 F.R. 15845, Dec. 23, 1965, as amended at 36 F.R. 9628, May 27, 1971]

§ 121.1072 Hydrogen cyanide.

The food additive hydrogen cyanide may be present as a residue in certain

§ 121.1236 2-(*p-tert*-Butylphenoxy)cyclohexyl 2-propynyl sulfite.

A tolerance of 30 parts per million is established for residues of the insecticide 2-(*p-tert*-butylphenoxy)cyclohexyl 2-propynyl sulfite in or on dried hops resulting from application of the insecticide to the raw agricultural commodity hops.

[35 F.R. 15991, Oct. 10, 1970]

§ 121.1237 Oleic acid derived from tall oil fatty acids.

The food additive oleic acid derived from tall oil fatty acids may be safely used in food and as a component in the manufacture of food-grade additives in accordance with the following prescribed conditions:

(a) The additive consists of purified oleic acid separated from refined tall oil fatty acids.

(b) The additive meets the following specifications:

(1) Specifications prescribed in "Food Chemicals Codex" for oleic acid, except that titer (solidification point) shall not exceed 13.5° C. and unsaponifiable matter shall not exceed 0.5 percent.

(2) The resin acid content does not exceed 0.01 percent as determined by ASTM Method D 1240–54 (1961).

(3) The requirements for absence of chick-edema factor as prescribed in § 121.1070.

(c) It is used or intended for use as follows:

(1) In foods as a lubricant, binder, and defoaming agent in accordance with good manufacturing practice.

(2) As a component in the manufacture of other food-grade additives.

(d) To assure safe use of the additive, the label and labeling of the additive and any premix thereof shall bear, in addition to the other information required by the act, the following:

(1) The common or usual name of the acid.

(2) The words "food grade" in juxtaposition with and equally as prominent as the name of the acid.

[36 F.R. 9628, May 27, 1971]

§ 121.1238 Synthetic fatty alcohols.

Synthetic fatty alcohols may be safely used in food and in the synthesis of food components in accordance with the following prescribed conditions:

(a) The food additive consists of any one of the following fatty alcohols: Hexyl, octyl, decyl, lauryl, myristyl, cetyl, and stearyl; manufactured by distillation of alcohols obtained by oxidation of organo-aluminums generated by the controlled reaction of low molecular weight trialkylaluminum with purified ethylene (minimum 99 percent by volume C_2H_4) and utilizing the hydrocarbon solvent as defined in paragraph (b) of this section, such that:

(1) Hexyl, octyl, decyl, lauryl, and myristyl alcohols contain not less than 99 percent of total alcohols and not less than 96 percent of straight chain alcohols. Any nonalcoholic impurities are primarily paraffins.

(2) Cetyl and stearyl alcohols contain not less than 98 percent of total alcohols and not less than 94 percent of straight chain alcohols. Any nonalcoholic impurities are primarily paraffins.

(3) The synthetic fatty alcohols contain no more than 0.1 weight percent of total diols as determined by a method available upon request from the Commissioner of Food and Drugs.

(b) The hydrocarbon solvent used in the process described in paragraph (a) of this section is a mixture of liquid hydrocarbons essentially paraffinic in nature derived from petroleum and refined to meet the specifications described in subparagraph (1) of this paragraph when subjected to the procedures described in subparagraphs (2) and (3) of this paragraph.

(1) The hydrocarbon solvent meets the following specifications:

(i) Boiling-point range: 175° C.-275° C.

(ii) Ultraviolet absorbance limits as follows:

Wavelength (millimicrons):	*Maximum absorbance per centimeter optical path length*
280–289	0.1[illegible]
290–299	.1[illegible]
300–359	.0[illegible]
360–400	.0[illegible]

(2) Use ASTM Method D–86 to determine boiling point range.

(3) The analytical method for determining ultraviolet absorbance limits is as follows:

(c) The labeling of the food additive shall contain adequate directions for its use to insure compliance with the requirements of paragraphs (a) and (b) of this section.

§ 121.2519 Defoaming agents used in the manufacture of paper and paperboard.

Defoaming agents may be safely used in the manufacture of paper and paperboard intended for use in packaging, transporting, or holding food in accordance with the following prescribed conditions:

(a) The defoaming agents are prepared from one or more of the subtances named in paragraph (d) of this section, subject to any prescribed limitations.

(b) The defoaming agents are used to prevent or control the formation of foam during the manufacture of paper and paperboard prior to and during the sheet-forming process.

(c) The quantity of defoaming agent or agents added during the manufacturing process shall not exceed the amount necessary to accomplish the intended technical effect.

(d) Substances permitted to be used in the formulation of defoaming agents include substances subject to prior sanctions or approval for such use and employed subject to the conditions of such sanctions or approvals, substances generally recognized as safe for use in food, substances generally recognized as safe for use in paper and paperboard, and substances listed in this paragraph, subject to the limitations, if any, prescribed.

(1) Fatty triglycerides, and the fatty acids, alcohols, and dimers derived therefrom:

Beef tallow.	Mustardseed oil.
Castor oil.	Palm oil.
Coconut oil.	Peanut oil.
Corn oil.	Rapeseed oil.
Cottonseed oil.	Ricebran oil.
Fish oil.	Soybean oil.
Lard oil.	Sperm oil.
Linseed oil.	Tall oil.

(2) Fatty triglycerides, and marine oils, and the fatty acids and alcohols derived therefrom (subparagraph (1) of this paragraph) reacted with one or more of the following, with or without dehydration, to form chemicals of the category indicated in parentheses:

Aluminum hydroxide (soaps).
Ammonia (amides).
Butanol (esters).
Butoxy-polyoxypropylene, molecular weight, 1,000–2,500 (esters).
Butylene glycol (esters).
Calcium hydroxide (soaps).
Diethanolamine (amides).
Diethylene glycol (esters).
Ethylene glycol (esters).
Ethylene oxide (esters and ethers).
Glycerin (mono- and diglycerides).
Hydrogen (hydrogenated compounds).
Hydrogen (amines).
Isobutanol (esters).
Isopropanol (esters).
Magnesium hydroxide (soaps).
Methanol (esters).
Morpholine (soaps).
Oxygen (air-blown oils).
Pentaerythritol (esters).
Polyoxyethylene, molecular weights 200, 300, 400, 600, 700, 1,000, 1,540, 1,580, 1,760, 4,000 (esters).
Polyoxypropylene, molecular weight 200–2,000 (esters).
Potassium hydroxide (soaps).
Propanol (esters).
Propylene glycol (esters).
Propylene oxide (esters).
Sodium hydroxide (soaps).
Sorbitol (esters).
Sulfuric acid (sulfated and sulfonated compounds).
Triethanolamine (amides and soaps).
Triisopropanolamine (amides and soaps).
Trimethylolethane (esters).
Zinc hydroxide (soaps).

(3) Miscellaneous:

Alcohols and ketone alcohols mixture (still-bottom product from C_{11}-C_{14} alcohol manufacturing process).
Amyl alcohol.
Butoxy polyethylene polypropylene glycol molecular weight 900–4,200.
Butoxy-polyoxypropylene molecular weight 1,000–2,500.
Butylated hydroxyanisole.
Butylated hydroxytoluene.
Calcium lignin sulfonate.
Capryl alcohol.
p-Chlorometacresol.
Cyclohexanol.
Diacetyltartaric acid ester of tallow monoglyceride.
Diethanolamine.
Diethylene triamine.
Di-(2-ethylhexyl) phthalate.
2,6-Dimethyl heptanol-4 (nonyl alcohol).
Dimethylpolysiloxane.
Di-*tert*-butyl hydroquinone.
Dodecylbenzene sulfonic acids.
Ethanol.
2-Ethylhexanol.
Ethylenediamine tetraacetic acid tetrasodium salt.
Formaldehyde.
Heavy oxo-fraction (a still-bottom product of iso-octyl alcohol manufacture, of approximate composition: Octyl alcohol 5 percent nonyl alcohol 10 percent, decyl and

higher alcohols 35 percent, esters 45 percent, and soaps 5 percent).
2-Heptadecenyl-4-methyl-4-hydroxymethyl-2-oxazoline.
Hexylene glycol (2-methyl-2-4-pentanediol).
12-Hydroxystearic acid.
Isobutanol.
Isopropanol.
Isopropylamine salt of dodecylbenzene sulfonic acid.
Kerosine.
Lanolin.
Methanol.
Methyl 12-hydroxystearate.
Methyl taurine-oleic acid condensate, molecular weight 486.
Mineral oil.
Mono-, di-, and triisopropanolamine.
Mono- and diisopropanolamine stearate.
Monobutyl ether of ethylene glycol.
Monoethanolamine.
Morpholine.
Myristyl alcohol.
Naphtha
β-Naphthol.
Nonylphenol.
Odorless light petroleum hydrocarbons.
Oleyl alcohol.
Petrolatum.
o-Phenylphenol.
Pine oil.
Polybutene, hydrogenated; complying with the identity prescribed under § 121.2511(b).
Polyethylene.
Polyethylene, oxidized (air-blown).
Polyoxyethylene (4 mols) decyl phosphate.
Polyoxyethylene (4 mols) di(2-ethyl hexanoate).
Polyoxyethylene (15 mols) ester of rosin.
Polyoxyethylene (3–15 mols) tridecyl alcohol.
Polyoxypropylene, molecular weight 200–2,000.
Polyoxypropylene-polyoxethylene condensate, minimum molecular weight 950.
Polyoxypropylene-ethylene oxide condensate of ethylene diamine, molecular weight 1,700–3,800.
Polyvinyl pyrrolidone, molecular weight 40,000.
Potassium distearyl phosphate.
Potassium pentachlorophenate.
Potassium trichlorophenate.
Rosins and rosin derivatives identified in § 121.2520(c)(5).
Silica.
Sodium alkyl (C_8–C_{18}) benzene-sulfonate.
Sodium dioctyl sulfosuccinate.
Sodium distearyl phosphate.
Sodium lauryl sulfate.
Sodium lignin sulfonate.
Sodium 2-mercaptobenzothiazole.
Sodium naphthalenesulfonic acid (3 mols) condensed with formaldehyde (2 mols).
Sodium orthophenylphenate.
Sodium pentachlorophenate.
Sodium petroleum sulfonate, molecular weight 440–450.
Sodium trichlorophenate.
Stearyl alcohol.
α-[p-(1,1,3,3-Tetramethylbutyl)phenyl-, p-nonylphenyl-, or p-dodecylphenyl]-*omega*-hydroxypoly(oxyethylene) produced by the condensation of 1 mole of p-alkylphenol (alkyl group is 1,1,3,3-tetramethylbutyl, a propylene trimer isomer, or a propylene tetramer isomer) with an average of 1.5–15 moles of ethylene oxide.
Tributoxyethyl phosphate.
Tributyl phosphate.
Tridecyl alcohol.
Triethanolamine.
Triethylene glycol di(2-ethyl hexanoate).
Tri- (2-ethylhexyl) phosphate.
Tristearyl phosphate.
Wax, petroleum, Type I and Type II.
Wax, petroleum (oxidized).
Wax (montan).

[30 F.R. 15845, Dec. 23, 1965, as amended at 32 F.R. 17656, Dec. 12, 1967; 33 F.R. 568, Jan. 17, 1968; 34 F.R. 6419, Apr. 12, 1969; 35 F.R. 5220, Mar. 28, 1970]

§ 121.2520 Adhesives.

(a) Adhesives may be safely used as components of articles intended for use in packaging, transporting, or holding food in accordance with the following prescribed conditions:

(1) The adhesive is prepared from one or more of the optional substances named in paragraph (c) of this section, subject to any prescribed limitations.

(2) The adhesive is either separated from the food by a functional barrier or used subject to the following additional limitations:

(i) *In dry foods.* The quantity of adhesive that contacts packaged dry food shall not exceed the limits of good manufacturing practice.

(ii) *In fatty and aqueous foods.* (*a*) The quantity of adhesive that contacts packaged fatty and aqueous foods shall not exceed the trace amount at seams and at the edge exposure between packaging laminates that may occur within the limits of good manufacturing practice.

(*b*) Under normal conditions of use the packaging seams or laminates will remain firmly bonded without visible separation.

(b) To assure safe usage of adhesives, the label of the finished adhesive container shall bear the statement "food-packaging adhesive."

(c) Subject to any limitations prescribed in this section and in any other regulation promulgated under section 409 of the act which prescribes safe con-

011

COMPONENTS OF ADHESIVES—Continued

Substances	*Limitations*
Diethylene glycol	
Dipentaerythritol	
Ethylene glycol	
Formaldehyde	
Fumaric acid	
Glycerin	
Hydrogen	
Isophthalic acid	
4,4′-Isopropylidenediphenol-epichlorohydrin (epoxy).	
4,4′-Isopropylidenediphenol-formaldehyde.	
Maleic anhydride	
Methyl alcohol	
Pentaerythritol	
Phthalic anhydride	
Polyethylene glycol	
Phenol-formaldehyde	
Phenyl o-cresol-formaldehyde.	
p-Phenylphenol-formaldehyde.	
Sulfuric acid	
Triethylene glycol	
Xylenol-formaldehyde	
Rosin salts (salts of wood, gum, and tall oil rosin, and the dimers thereof, decarboxylated rosin, disproportionated rosin, hydrogenated rosin):	
Aluminum	
Ammonium	
Calcium	
Magnesium	
Potassium	
Sodium	
Zinc	
Rosin, gasoline-insoluble fraction.	
Rubber hydrochloride polymer.	
Rubber latex, natural	
Salicylic acid	For use as preservative only.
Sandarac	
Sebacic acid	
Shellac	
Sodium alkyl (C_3-C_{18} aliphatic) benzenesulfonate.	
Sodium aluminum pyrophosphate.	
Sodium aluminum sulfate.	
Sodium bisulfate	
Sodium calcium silicate	
Sodium capryl polyphosphate.	
Sodium carboxymethylcellulose.	
Sodium chlorate	
Sodium chlorite	

COMPONENTS OF ADHESIVES—Continued

Substances	*Limitations*
Sodium chromate	
Sodium decylsulfate	
Sodium dehydroacetate	For use as preservative only.
Sodium di-(2-ethylhexoate).	
Sodium di(2-ethylhexyl) pyrophosphate.	
Sodium dihexylsulfosuccinate.	
Sodium diisobutylphenoxydiethoxyethyl sulfonate.	
Sodium diisobutylphenoxymonoethoxyethyl sulfonate.	
Sodium diisopropyl- and triisopropylnaphthalenesulfonate.	
Sodium dimethyldithiocarbamate.	
Sodium dioctylsulfosuccinate.	
Sodium n-dodecylpolyethoxy (50 moles) sulfate.	
Sodium ethylene ether of nonylphenol sulfate.	
Sodium 2-ethylhexyl sulfate.	
Sodium fluoride	For use only as bonding agent for aluminum foil, stabilizer, or preservative. Total fluoride for all sources not to exceed 1 percent by weight of the finished adhesive.
Sodium formaldehyde sulfoxylate.	
Sodium formate	
Sodium heptadecylsulfate.	
Sodium hypochlorite	
Sodium isododecylphenoxypolyethoxy (40 moles) sulfate.	
Sodium N-lauroyl sarcosinate.	
Sodium metaborate	
Sodium α-naphthalene sulfonate.	
Sodium nitrate	
Sodium nitrite	
Sodium oleoyl isopropanolamide sulfosuccinate.	
Sodium pentachlorophenate.	For use as preservative only.
Sodium perborate	
Sodium persulfate	

COMPONENTS OF ADHESIVES—Continued

Substances	Limitations
Sodium o-phenylphenate	For use as preservative only.
Sodium polyacrylate	
Sodium polymethacrylate	
Sodium polystyrene sulfonate.	
Sodium salicylate	For use as preservative only.
Sodium tetradecylsulfate.	
Sodium thiocyanate	
Sodium bis-tridecylsulfosuccinate.	
Sodium xylene sulfonate	
Sorbitan monooleate	
Sorbitan monopalmitate	
Sorbitan monostearate	
Soribtan trioleate	
Sorbitan tristearate	
Soybean oil, epoxidized	
Spermaceti wax	
Sperm oil wax	
Stannous oleate	For use only as a catalyst for polyurethane resins.
Stannous stearate	
Starch hydrolysates	
Starch or starch modified by one or more of the treatments described in §§ 121.1031 and 121.2506.	
Starch, reacted with a urea - formaldehyde resin.	
Starch, reacted with formaldehyde.	
Stearamide (stearic acid amide).	
Stearic acid	
Stearic acid-chromic chloride complex.	
Stearyl-cetyl alcohol, technical grade, approximately 65 percent–80 percent stearyl and 20 percent–35 percent cetyl.	
Strontium salicylate	
Styrenated phenol	
Styrene-maleic anhydride copolymer, ammonium or potassium salt.	
Styrene-maleic anhydride copolymer (partially methylated) sodium salt.	
Styrene-methacrylic acid copolymer, potassium salt.	
Sucrose acetate isobutyrate.	
Sucrose benzoate	
Sucrose octaacetate	
Sulfonated octadecylene (sodium form).	

COMPONENTS OF ADHESIVES—Continued

Substances	Limitations
Sulfur	
Tall oil	
Tall oil fatty acids, linoleic and oleic.	
Tall oil fatty acid methyl ester.	
Tall oil, methyl ester	
Tall oil pitch	
Tall oil soaps	
Tallow alcohol (hydrogenated).	
Tallow amine, secondary (hexadecyl, octadecyl), of hard tallow.	
Tallow, blown (oxidized)	
Tallow, propylene glycol ester.	
Terpene resins (α- and β-pinene) homopolymers, copolymers, and condensates with phenol, formaldehyde, coumarone, and/or indene.	
Terphenyl	
Terphenyl, hydrogenated	
Terpineol	
Tetraethylene pentamine	
Tetraethylthiuram disulfide.	
Tetrahydrofuran	
Tetrahydrofurfuryl alcohol.	
Tetra-isopropyl titanate	
α-[p-(1,1,3,3 - Tetramethylbutyl) phenyl]-*omega*-hydroxypoly - (oxyethylene) produced by the condensation of 1 mole of p-(1,1,3,3-tetramethylbutyl) phenol with an average of 1–40 moles of ethylene oxide.	
Tetrakis[methylene (3,5-di - *tert* - butyl-4-hydroxy - hydrocinnamate)] methane.	
α-[p-(1,1,3,3 - Tetramethylbutyl) phenyl]-*omega* - hydroxypoly (oxyethylene) mixture of dihydrogen phosphate and monohydrogen phosphate esters and their sodium, potassium, and ammonium salts having a poly (oxyethylene) content averaging 6–9 or 40 moles.	
Tetramethyl decanediol	
Tetramethyl decynediol	
Tetramethyl decynediol plus 1–30 moles of ethylene oxide.	
Tetramethylthiuram monosulfide.	

(b) The substances permitted are as follows:

List of substances	*Limitations*
Copper-8-quinolinolate	
Mineral spirits	
Paraffin wax	Used singly or in combination so as to constitute not less than 50% of the solids.
Petroleum hydrocarbon resin, produced by the homo- and copolymerization of dienes and olefins of the aliphatic, alicyclic, and monobenzenoid arylalkene type from distillates of cracked petroleum stocks.	Do.
Pentachlorophenol and its sodium salt	Not to exceed 50 p.p.m. in the treated wood, calculated as pentachlorophenol.
Rosins and rosin derivatives	As provided in § 121.2592.
Zinc salt of sulfonated petroleum	

§ 121.2557 Defoaming agents used in coatings.

The defoaming agents described in this section may be safely used as components of articles intended for use in producing, manufacturing, packing, processing, preparing, treating, packaging, transporting, or holding food, subject to the provisions of this section.

(a) The defoaming agents are prepared as mixtures of substances described in paragraph (d) of this section.

(b) The quantity of any substance employed in the formulation of defoaming agents does not exceed the amount reasonably required to accomplish the intended physical or technical effect in the defoaming agents or any limitation further provided.

(c) Any substance employed in the production of defoaming agents and which is the subject of a regulation in this Subpart F conforms with any specification in such regulation.

(d) Substances employed in the formulation of defoaming agents include:

(1) Substances generally recognized as safe in food.

(2) Substances subject to prior sanction or approval for use in defoaming agents and used in accordance with such sanction or approval.

(3) Substances identified in this subparagraph and subject to such limitations as are provided:

List of substances	Limitations
n-Butyl alcohol	
tert-Butyl alcohol	
Butyl stearate	
Castor oil, sulfated, ammonium, potassium, or sodium salt.	
Cetyl alcohol	
Cyclohexane	
Cyclohexanol	
Diethylene glycol monolaurate	
Diethylene glycol monostearate	
Dimers and trimers of unsaturated C_{18} fatty acids derived from: Animal and vegetable fats and oils. Tall oil.	For use only at levels not to exceed 0.1% by weight of total coating solids.
Dimethylpolysiloxane	
Dipropylene glycol	
Ethyl alcohol	
Fats and oils derived from animal, marine, or vegetable sources.	
Fatty acids derived from animal, marine, or vegetable fats and oils, and salts of such acids, single or mixed, as follows: Aluminum. Ammonium. Calcium. Magnesium. Potassium. Sodium. Zinc.	
Formaldehyde	For use as preservative of defoamer only.
Glyceryl mono-12-hydroxystearate.	
Glyceryl monostearate	
Hexane	
Hexylene glycol (2-methyl-2,4-pentanediol).	
Isobutyl alcohol	
Isopropyl alcohol	
Kerosene	
Lecithin hydroxylated	
Methyl alcohol	
Methylcellulose	
Methyl esters of fatty acids derived from animal, marine, or vegetable fats and oils.	
Methyl oleate	
Methyl palmitate	
Mineral oil.	
Mustardseed oil, sulfated, ammonium, potassium, or sodium salt.	
Myristyl alcohol	
Naphtha	
β-Naphthol	For use as preservative of defoamer only.
Nonyl phenol	
Odorless light petroleum hydrocarbons.	As defined in § 121.2594.
Oleic acid, sulfated, ammonium, potassium, or sodium salt.	
Parachlorometacresol	For use as preservative of defoamer only.
Peanut oil, sulfated, ammonium, potassium, or sodium salt.	
Petrolatum	

List of substances	Limitations
Pine oil	
Polyacrylic acid, sodium salt	As a stabilizer and thickener in defoaming agents containing dimethylpolysiloxane.
Polyethylene	
Polyethylene, oxidized	
Polyethylene glycol (200) dilaurate.	
Polyethylene glycol (400) dioleate.	
Polyethylene glycol (600) dioleate.	
Polyethylene glycol (400) esters of coconut oil fatty acids.	
Polyethylene glycol (400) monooleate.	
Polyethylene glycol (600) monooleate.	
Polyethylene glycol (600) monoricinoleate.	
Polyethylene glycol (400) monostearate.	
Polyoxybutylene-polyoxypropylene-polyoxyethylene glycol (min. mol. wt. 3700).	
Polyoxyethylated (min. 3 mols) cetyl alcohol.	
Polyoxyethylated (min. 5 mols) oleyl alcohol.	
Polyoxyethylated (min. 1.5 mols) tridecyl alcohol.	
Polyoxyethylene (min. 15 mols) ester of rosin.	
Polyoxyethylene (min. 8 mols) monooleate.	
Polyoxyethylene (40) stearate	
Polyoxypropylated (min. 20 mols) butyl alcohol.	
Polyoxypropylene glycol (min. mol. wt. 200).	
Polyoxypropylene (min. 20 mols) oleate butyl ether.	
Polyoxypropylene-polyoxyethylene glycol (min. mol. wt. 1,900).	
Polyoxypropylene (min. 40 mols) stearate butyl ether.	
Potassium pentachlorophenate	For use as preservative of defoamer only.
Potassium trichlorophenate	For use as preservative of defoamer only.
Propylene glycol monoester of soybean oil fatty acids.	
Propylene glycol monoester of tallow fatty acids.	
Ricebran oil, sulfated, ammonium, potassium, or sodium salt.	
Rosins and rosin derivatives	As provided in § 121.2592.
Silica.	
Sodium 2-mercaptobenzothiazole.	For use as preservative of defoamer only.
Sodium pentachlorophenate	For use as preservative of defoamer only.
Sodium trichlorophenate	For use as preservative of defoamer only.
Sorbitan tristearate	
Sperm oil, sulfated, ammonium, potassium, or sodium salt.	
Stearyl alcohol	
Tall oil fatty acids	
Tallow fatty acids, hydrogenated or sulfated.	
Tallow, sulfated, ammonium, potassium, or sodium salt.	
Triethanolamine	
Triisopropanolamine	
Waxes, petroleum	

(e) The defoaming agents are used as follows:

(1) The quantity of defoaming agent or agents used shall not exceed the amount reasonably required to accomplish the intended effect, which is to prevent or control the formation of foam.

(2) The defoaming agents are used in the preparation and application of coatings for paper and paperboard.

[30 F.R. 15845, Dec. 23, 1965, as amended at 31 F.R. 15570, Dec. 10, 1966; 32 F.R. 4060, Mar. 15, 1967; 32 F.R. 14552, Oct. 19, 1967; 34 F.R. 7374, May 7, 1969; 34 F.R. 12089, July 18, 1969]

§ 121.2558 Isoparaffinic petroleum hydrocarbons, synthetic.

Isoparaffinic petroleum hydrocarbons, synthetic, may be safely used in the production of nonfood articles intended for use in producing, manufacturing, packing, processing, preparing, treating, packaging, transporting, or holding food, subject to the provisions of this section.

(a) The isoparaffinic petroleum hydrocarbons, produced by synthesis from petroleum gases, consist of a mixture of liquid hydrocarbons meeting the following specifications:

Boiling point 145°–500° F., as determined by A.S.T.M. Method D–86.

Ultraviolet absorbance:

260–319 millimicrons—1.5 maximum.

320–329 millimicrons—0.08 maximum.

330–350 millimicrons—0.05 maximum.

Nonvolatile residue 0.002 gram per 100 milliliters maximum.

Synthetic isoparaffinic petroleum hydrocarbons containing antioxidants shall meet the specified ultraviolet absorbance limits after correction for any absorbance due to the antioxidants. The ultraviolet absorbance shall be determined by the procedure described for application to mineral oil under "Specifications" on page 66 of the Journal of the Association of Official Agricultural Chemists, Vol. 45 (February 1962), disregarding the last sentence of that procedure For hydrocarbons boiling below 250° F., the nonvolitile residue shall be determined by A.S.T.M. procedure D–1353; for those boiling above 250° F., A.S.T.M. procedure D–381 shall be used.

(b) Isoparaffinic petroleum hydrocarbons may contain antioxidants authorized for use in food in an amount not to exceed that reasonably required to accomplish the intended technical effect.

(c) Isoparaffinic petroleum hydrocarbons are used in the production of nonfood articles. The quantity used shall not exceed the amount reasonably required to acomplish the intended technical effect, and the residual remaining in the finished article shall be the minimum amount reasonably attainable.

§ 121.2559 Xylene-formaldehyde resins condensed with 4,4′-isopropylidenediphenol-epichlorohydrin epoxy resins.

The resins identified in paragraph (a) of this section may be safely used as a food-contact coating for articles intended for use in contact with food, in accordance with the following prescribed conditions.

(a) The resins are produced by the condensation of xylene-formaldehyde resin and 4,4′-isopropylidenediphenol-epichlorohydrin epoxy resins, to which may have been added certain optional adjuvant substances required in the production of the resins or added to impart desired physical and technical properties. The optional adjuvant substances may include resins produced by the condensation of allyl ether of mono-, di-, or trimethylol phenol and capryl alcohol and also may include substances identified in § 121.2514(b)(3), with the exception of paragraph (b)(3)(xxxi) and (xxxii) of that section.

(b) The resins identified in paragraph (a) of this section may be used as a food-contact coating for articles intended for contact at temperatures not to exceed 160° F. with food of types I, II, VI–A and B, and VIII described in table 1 of § 121.2526(c) provided that the coating in the finished form in which it is to contact food meets the following extractives limitations when tested by the methods provided in § 121.2514(e):

(1) The coating when extracted with distilled water at 180° F. for 24 hours yields total extractives not to exceed 0.05 milligram per square inch of food-contact surface.

(2) The coating when extracted with 8 percent (by volume) ethyl alcohol in distilled water at 160° F. for 4 hours yields total extractives not to exceed 0.05 milligram per square inch of food-contact surface.

(c) The resins identified in paragraph (a) of this section may be used as a food-contact coating for articles intended for contact at temperatures not to exceed room temperature with food of type VI–C described in table 1 of § 121.2526(c) provided the coating in the finished form in which it is to contact food meets the following extractives limitations when tested by the methods provided in § 121.2514(e):

(1) The coating when extracted with distilled water at 180° F. for 24 hours yields total extractives not to exceed 0.05 milligram per square inch of food-contact surface.

(2) The coating when extracted with 50 percent (by volume) ethyl alcohol in distilled water at 180° F. for 24 hours yields total extractives not to exceed 0.05 milligram per square inch.

[34 F.R. 12089, July 18, 1969]

§ 121.2560 Poly-1,4,7,10,13-pentaaza-15-hydroxyhexadecane.

Poly - 1,4,7,10,13-pentaaza-15-hydroxyhexadecane may be safely used as a retention aid employed prior to the sheet-forming operation in the manufacture of paper and paperboard intended for use in contact with food in an amount not to exceed that necessary to accomplish the intended physical or technical effect and not to exceed 6 pounds per ton of finished paper or paperboard.

§ 121.2561 Esters of stearic and palmitic acids.

The ester stearyl palmitate or palmityl stearate or mixtures thereof may be safely used as adjuvants in food-packaging materials when used in accordance with the following prescribed conditions:

(a) They are used or intended for use as plasticizers or lubricants in polystyrene intended for use in contact with food.

(b) They are added to the formulated polymer prior to extrusion.

(c) The quantity used shall not exceed that required to accomplish the intended technical effect.

§ 121.2562 Rubber articles intended for repeated use.

Rubber articles intended for repeated use may be safely used in producing, manufacturing, packing, processing, preparing, treating, packaging, transporting, or holding food, subject to the provisions of this section.

(a) The rubber articles are prepared from natural and/or synthetic polymers and adjuvant substances as described in paragraph (c) of this section.

(b) The quantity of any substance employed in the production of rubber articles intended for repeated use shall not exceed the amount reasonably required to accomplish the intended effect in the ubber article and shall not be intended o accomplish any effect in food.

(c) Substances employed in the preparation of rubber articles include the following, subject to any limitations prescribed:

(1) Substances generally recognized as safe for use in food or food packaging.

(2) Substances used in accordance with the provisions of a prior sanction or approval.

(3) Substances that by regulation in this Part 121 may be safely used in rubber articles, subject to the provisions of such regulation.

(4) Substances identified in this subparagraph, provided that any substance that is the subject of a regulation in this Subpart F conforms with any specification in such regulation.

(i) *Elastomers.*

Acrylonitrile-butadiene copolymer.
Butadiene-acrylonitrile-ethylene glycol dimethacrylate copolymers containing not more than 5 weight percent of polymer units derived from ethylene glycol dimethacrylate.
Butadiene-acrylonitrile-methacrylic acid copolymer.
Butadiene-styrene-methacrylic acid copolymer.
Chloroprene polymers.
Chlorotrifluoroethylene-vinylidene fluoride copolymer.
Ethylene-propylene copolymer elastomers which may contain not more than 5 weight-percent of total polymer units derived from 5-methylene-2-norbornene and/or 5-ethylidine-2-norbornene.
Ethylene-propylene-dicyclopentadiene copolymer.
Ethylene-propylene-1,4-hexadiene copolymers containing no more than 8 weight percent of total polymer units derived from 1,4-hexadiene.
Isobutylene-isoprene copolymer.
Polybutadiene.
Polyisoprene.
Polyurethane resins derived from reactions of diphenylmethane diisocyanate with adipic acid and 1,4-butanediol.
Rubber, natural.
Silicone basic polymers as described in ASTM D-1418-61T:
Silicone (Si) elastomers containing methyl groups.
Silicone (Psi) elastomers containing methyl and phenyl groups.
Silicone (Vsi) elastomers containing methyl and vinyl groups.
Silicone (Fsi) elastomers containing methyl and fluorine groups.
Silicone (PVsi) elastomers containing phenyl, methyl, and vinyl groups.
Styrene-butadiene copolymer.
Vinylidene fluoride-hexafluoropropylene copolymers (minimum number average molecular weight 70,000 as determined by osmotic pressure in methyl ethyl ketone).
Vinylidene fluoride - hexafluoropropylene-tetrafluoroethylene copolymers (minimum number average molecular weight 100,000 as determined by osmotic pressure in methyl ethyl ketone).

(ii) *Vulcanization materials—(a) Vulcanizing agents.*

4,4′-Bis(aminocyclohexyl)methane carbamate for use only as cross-linking agent in the vulcanization of vinylidene fluoridehexafluoropropylene copoylmyer and vinylidene fluoride-hexafluoropropylene-tetrafluoroethylene copolymer elastomers identified under subdivision (i) of this subparagraph and limited to use at levels not to exceed 2.4 percent by weight of such copolymers.
Hexamethylenediamine carbamate for use only as cross-linking agent in the vulcanization of vinylidene fluoride-hexafluoropropylene copolymer and vinylidene fluoride-hexafluoropropylene-tetrafluoroethylene copolyfer elastomers identified under subdivision (i) of this subparagraph and limited to use at levels not to exceed 1.5 percent by weight of such copolymers.
Sulfur, ground.

(*b*) *Accelerators (total not to exceed 1.5 percent by weight of rubber product).*

2-Benzothiazyl - *N,N*-diethylthiocarbamyl-sulfide.
Benzoyl peroxide.
1,3 - Bis(2-benzothiazolylmercaptomethyl) urea.
N-tert-Butyl-2-benzothiazole sulfenamide.
Butyraldehyde-aniline resin (iodine number 670–705).
Carbon disulfide - 1,1′ - methylenedipiperidine reaction product.
Copper dimethyldithiocarbamate.
N-Cyclohexyl-2-benzothiazole sulfenamide.
Dibenzoyl-*p*-quinone dioxime.
Dibenzylamine.
Di-*tert*-butyl peroxide.
Dibutyl xanthogen disulfide.
2,4-Dichlorobenzoyl peroxide.
Dicumyl peroxide.
N,N-Dimethylcyclohexylamine salt of dibutyldithiocarbamic acid.
2,6-Dimethylmorpholine thiobenzothiazol.
Dipentamethylenethiuram tetrasulfide.
Diphenylguanidine.
Diphenylguanidine phthalate.
1,3-Diphenyl-2-thiourea.
2,2′-Dithiobis[benzothiazole].
4,4′-Dithiodimorpholine.
N,N′-Di-*o*-tolylguanidine.
Di-*o*-tolylguanidine salt of pyrocatechol borate.
Ethylenediamine carbamate.
Heptaldehyde-aniline resin (iodine number 430–445).

Hexamethylenetetramine.
2-Mercaptobenzothiazole.
2-Mercaptoimidazoline.
2-Mercaptothiazoline.
N-Oxydiethylene - benzothiazole-2-sulfenamide.
Piperidinium pentamethylenedithiocarbamate.
Potassium pentamethylenedithiocarbamate.
p-Quinone dioxime.
Sodium dibutyldithiocarbamate.
Sodium dimethyldithiocarbamate.
Stannous oleate for use only as an accelerator for silicone elastomers.
Tetrabutylthiuram monosulfide.
Tetraethylthiuram disulfide.
(1, 1, 4, 4-Tetramethyltetramethylene) bis [*tert*-butyl peroxide].
Tetramethylthiuram monosulfide.
Thiram (tetramethylthiuram disulfide).
Triallyl cyanurate.
Triethylenetetramine.
1,3,5-Triethyl-hexahydro-*s*-triazine (triethyltrimethylenetriamine).
Triphenylguanidine.
Zinc butyl xanathate.
Zinc dibenzyl dithiocarbamate.
Zinc dibutyldithiocarbamate.
Zinc diethyldithiocarbamate.
Zinc 2-mercaptobenzothiazole.
Ziram (zinc dimethyldithiocarbamate).

(c) *Retarders (total not to exceed 10 percent by weight of rubber product).*

Cyanoguanidine.
Phthalic anhydride.
Salicylic acid.

(d) *Activators (total not to exceed 5 percent by weight of rubber product except magnesium oxide may be used at higher levels).*

Diethylamine.
Fatty acid amines, mixed.
Fatty acids.
Magnesium carbonate.
Magnesium oxide, light and heavy.
Oleic acid, dibutylamine salt (dibutylammonium oleate).
Stannous chloride.
Tall oil fatty acids.
Tetrachloro-*p*-benzoquinone.
Triethanolamine.
Zinc salts of fatty acids.

(iii) *Antioxidants and antiozonants (total not to exceed 5 percent by weight of rubber product).*

Aldol-α-naphthylamine.
Alkylated (C_4 and/or C_8) phenols.
BHT (butylated hydroxytoluene).
Butylated, styrenated cresols identified in § 121.2566(b).
4,4'-Butylidinebis(6-*tert*-butyl-*m*-cresol).
N-Cyclohexyl-*N*'-phenylphenylenediamine.
p,p'-Diaminodiphenylmethane.
2,5-Di-*tert*-amylhydroquinone.
Diaryl-*p*-phenylenediamine, where the aryl group may be phenyl, tolyl, or xylyl.
2,6-Di-*tert*-butyl-*p*-phenylphenol.
1,2-Dihydro-2,2,4 - trimethyl - 6 - dodecylquinoline.
1,2-Dihydro-2,2,4 - trimethyl - 6 - ethoxyquinoline.
1,2-Dihydro-2,2,4-trimethyl - 6 - phenylquinoline.
4,4'-Dimethoxydiphenylamine.
4,6-Dinonyl-*o*-cresol.
N,N'-Dioctyl-*p*-phenylenediamine.
Diphenylamine-acetone resin.
Diphenylamine - acetone - formaldehyde resin.
N,N'-Diphenylethylenediamine.
N,N'-Disalicylalpropylenediamine.
N,N'-Di-*o*-tolylethylenediamine.
Hydroquinone monobenzyl ether.
Isopropoxydiphenylamine.
N - Isopropyl - *N*' - phenyl - *p* - phenylenediamine.
2,2' - Methylenebis(6 - *tert* - butyl-4-ethylphenol).
2,2' - Methylenebis(4 - methyl - 6 - *tert*. butylphenol).
2,2'-Methylenebis(4-methyl - 6 - nonylphenol).
2,2' - Methylenebis(4 - methyl - 6 - *tert*. octylphenol).
Monooctyl- and dioctyldiphenylamine.
N,N'-Di-β-naphthyl-*p*-phenylenediamine.
Phenyl-α-naphthylamine.
Phenyl-β-naphthylamine.
Phenyl-β-naphthylamine-acetone aromatic amine resin (average molecular weight 600; nitrogen content 5.3 percent).
o- and *p*-Phenylphenol.
Polybutylated (mixture) 4,4'-isopropylidenediphenol.
Sodium pentachlorophenate.
Styrenated cresols produced when 2 moles of styrene are made to react with 1 mole of a mixture of phenol and *o*-, *m*-, and *p*-cresols so that the final product has a Brookfield viscosity at 25° C. of 1400 to 1700 centipoises.
Styrenated phenol.
4,4'-Thiobis (6-*tert*-butyl-*m*-cresol).
Toluene-2,4-diamine.
N-*o*-Tolyl-*N*'-phenyl-*p*-phenylenediamine.
p(*p*-Tolylsulfanilamide) diphenylamine.
Tri(mixed mono- and dinonylphenyl) phosphite.
Tri(nonylphenyl) phosphite-formaldehyde resins produced when 1 mole of tri(nonylphenyl) phosphite is made to react with 1.4 moles of formaldehyde or produced when 1 mole of nonylphenol is made to react with 0.36 mole of formaldehyde and the reaction product is then further reacted with 0.33 mole of phosphorus trichloride. The finished resins have a minimum viscosity of 20,000 centipoises at 25° C., as determined by LV-series Brookfield viscometer (or equivalent) using a No. 4 spindle at 12 r.p.m., and have an organic phosphorus content of 4.05 to 4.15 percent by weight.

(8) *Determination of ultraviolet-absorbing extractives.* (i) A distilled water solution containing 1.0 part per million of *p*-methoxyphenol (melting point 54° C.–56° C. Eastman grade or equivalent) shall be scanned in the region 360 to 220 mμ in 5-centimeter silica spectrophotometric absorption cells versus a distilled water reference. The absorbance at the wavelength of maximum absorbance (should be about 285 mμ) is about 0.11 but must be not less than 0.08 nor more than 0.14. This test shall be run in duplicate. For the purpose of ascertaining compliance with the limitations prescribed in paragraph (b) (3) and (4) of this section, the absorbance obtained on the extracts according to subdivision (ii) of this subparagraph shall be multiplied by a correction factor, calculated as follows:

$$\frac{0.11}{\text{Average of duplicate } p\text{-methoxyphenol absorbance determinations according to this subdivision (i) of this subparagraph.}} = \text{Correction factor for ultraviolet absorbers test.}$$

(ii) An aliquot of the extract that has been exposed under the conditions specified in subparagraph (5) of this paragraph is scanned in the wavelength region 360 to 220 mμ versus the appropriate solvent reference in matched 5-centimeter silica spectrophotometric absorption cells. The height of any absorption peak shall be measured, corrected for the blank as determined in subparagraph (4)(iii) of this paragraph, and multiplied by the correction factor determined according to subdivision (i) of this subparagraph.

(d) In accordance with good manufacturing practice, finished semirigid and rigid acrylic and modified acrylic plastics intended for repeated use in contact with food shall be thoroughly cleansed prior to their first use in contact with food.

[30 F.R. 15846, Dec. 23, 1965, as amended at 34 F.R. 5292, Mar. 15, 1969; 34 F.R. 18384, Nov. 18, 1969]

§ 121.2592 Rosins and rosin derivatives.

The rosins and rosin derivatives identified in paragraph (a) of this section may safely be used in the manufacture of articles or components of articles intended for use in producing, manufacturing, packing, processing, preparing treating, packaging, transporting, or holding food, subject to the provisions of this section.

(a) The rosins and rosin derivatives are identified as follows:

(1) Rosins:

(i) Gum rosin, refined to color grade of K or paler.

(ii) Wood rosin, refined to color grade of K or paler.

(iii) Tall oil rosin, refined to color grade of K or paler.

(iv) Dark tall oil rosin, a fraction resulting from the refining of tall oil rosin produced by multicolumnar distillation of crude tall oil to effect removal of fatty acids and pitch components and having a saponification number of from 110–135 and 32 percent–44 percent rosin acids.

(v) Dark wood rosin, all or part of the residue after the volatile terpene oils are distilled from the oleoresin extracted from pine wood.

(2) Modified rosins manufactured from rosins identified in subparagraph (1) of this paragraph:

(i) Partially hydrogenated rosin, catalytically hydrogenated to a maximum refractive index of 1.5012 at 100° C., and a color of WG or paler.

(ii) Fully hydrogenated rosin, catalytically hydrogenated to a maximum dehydroabietic acid content of 2 percent, a minimum drop-softening point of 79° C., and a color of X or paler.

(iii) Partially dimerized rosin, dimerized by sulfuric acid catalyst to a drop-softening point of 95° C.–105° C., and a color of WG or paler.

(iv) Fully dimerized rosin, dimerized by sulfuric acid catalyst, and from which sufficient nondimerized rosin has been removed by distillation to achieve a minimum drop-softening point of 143° C., and a color of H or paler.

(v) Disproportionated rosin, catalytically disproportionated to a minimum dehydroabietic acid content of 35 percent, a maximum abietic acid content of 1 percent, a maximum content of substituted phenanthrenes (as retene) of 0.25 percent, and a color of WG or paler.

(3) Rosin esters manufactured from rosins and modified rosins identified in

subparagraphs (1) and (2) of this paragraph:

(i) Glycerol ester of wood rosin purified by steam stripping to have an acid number of 3 to 9, a drop-softening point of 88° C.–96° C., and a color of N or paler.

(ii) Glycerol ester of partially hydrogenated wood rosin, having an acid number of 3 to 10, a drop-softening point of 79° C.–88° C., and a color of N or paler.

(iii) Glycerol ester of partially dimerized rosin, having an acid number of 3 to 8, a drop-softening point of 109° C.–119° C., and a color of M or paler.

(iv) Glycerol ester of fully dimerized rosin, having an acid number of 5 to 16, a drop-softening point of 165° C.–175° C., and a color of H or paler.

(v) Glycerol ester of maleic anhydride-modified wood rosin, having an acid number of 30 to 40, a drop-softening point of 138° C.–146° C., a color of M or paler, and a saponification number less than 280.

(vi) Methyl ester of rosin, partially hydrogenated, purified by steam stripping to have an acid number of 4 to 8, a refractive index of 1.5170 to 1.5205 at 20° C., and a viscosity of 23 to 66 poises at 25° C.

(vii) Pentaerythritol ester of wood rosin, having an acid number of 6 to 16, a drop-softening point of 109° C.–116° C., and a color of M or paler.

(viii) Pentaerythritol ester of partially hydrogenated wood rosin, having an acid number of 7 to 18, a drop-softening point of 102° C.–110° C., and a color of K or paler.

(ix) Pentaerythritol ester of maleic anhydride-modified wood rosin, having an acid number of 8 to 16, a drop-softening point of 154° C.–162° C., a color of M or paler, and having a saponification number less than 280.

(x) Pentaerythritol ester of maleic anhydride-modified wood rosin, having an acid number of 9 to 16, a drop-softening point of 130° C.–140° C., a color of N or paler, and having a saponification number less than 280.

(xi) Pentaerythritol ester of maleic anhydride-modified wood rosin, having an acid number of 134 to 145, a drop-softening point of 127° C.–137° C., a color of M or paler, and having a saponification number less than 280.

(xii) Pentaerythritol ester of maleic anhydride-modified wood rosin, having an acid number of 30 to 40, a drop-softening point of 131° C.–137° C., a color of N or paler, and having a saponification number less than 280.

(xiii) Pentaerythritol ester of maleic anhydride-modified wood rosin, further modified by reaction with 4,4'-isopropylidenediphenol-formaldehyde condensate, having an acid number of 10 to 22, a drop-softening point of 162° C.–172° C., a color of K or paler, a saponification number less than 280, and a maximum ultraviolet absorbance of 0.14 at 296 mμ (using a 1-centimeter cell and 200 milligrams of the rosin ester per liter of solvent consisting of ethyl alcohol made alkaline by addition of 0.1 percent of potassium hydroxide).

(xiv) Mixed methyl and pentaerythritol ester of maleic anhydride-modified wood rosin, having an acid number of 73 to 83, a drop-softening point of 113° C.–123° C., a color of M or paler, and a saponification number less than 280.

(xv) Triethylene glycol ester of partially hydrogenated wood rosin, having an acid number of 2 to 10, a color of K or paler, and a viscosity of 350 to 425 seconds Saybolt at 100° C.

(xvi) Glycerol ester of maleic anhydride-modified wood rosin, having an acid number of 17 to 23, a drop-softening point of 136° C.–140° C., a color of M or paler, and a saponification number less than 280. For use only in cellophane complying with § 121.2507.

(xvii) Citric acid-modified glycerol ester of rosin, having an acid number less than 20, a drop-softening point of 105° C.–115° C., and a color of K or paler. For use only as a blending agent in coatings for cellophane complying with § 121.2507.

(xviii) Glycerol ester of tall oil rosin, purified by steam stripping to have an acid number of 5–12, a softening point of 80° C.–88° C., and a color of N or paler.

(xix) Glycerol ester of maleic anhydride-modified tall oil rosin, having an acid number of 30 to 40, a drop-softening point of 141° C.–146° C., a color of N or paler, and a saponification number less than 280.

(xx) Glycerol ester of disproportionated tall oil rosin, having an acid number of 5 to 10, a drop-softening point of 84° C.–93° C., a color of WG or paler, and a saponification number less than 180.

(4) Rosin salts and sizes—Ammonium, calcium, potassium, sodium, or zinc salts

of rosin manufactured by the partial or complete saponification of any one of the rosins or modified rosins identified in subparagraphs (1) and (2) of this paragraph, or blends thereof, and with or without modification by reaction with one or more of the following:

(i) Formaldehyde.

(ii) Fumaric acid.

(iii) Maleic anhydride.

(iv) Saligenin.

(b) The quantity used shall not exceed the amount reasonably required to accomplish the intended technical effect.

(c) The use in any substance or article that is the subject of a regulation in this Subpart F shall conform with any specifications and limitations prescribed by such regulation for the finished form of the substance or article.

(d) The provisions of this section are not applicable to rosins and rosin derivatives identified in § 121.2514(b)(3)(v) and used in resinous and polymeric coatings complying with § 121.2514.

(e) The provisions of this section are not applicable to rosins and rosin derivatives identified in § 121.2520(c)(5) and used in defoaming agents complying with § 121.2519, food-packaging adhesives complying with § 121.2520, and rubber articles complying with § 121.2562.

(f) The analytical methods for determining whether rosins and rosin derivatives conform to the specifications prescribed in paragraph (a) of this section are as follows:

(1) Color: Color shall be determined by ASTM Method D 509–55.

(2) Refractive index: Refractive index shall be determined by ASTM Method D 1747–62.

(3) Acid number: Acid number shall be determined by ASTM Method D 465–59.

(4) Viscosity: Viscosity in poises shall be determined by ASTM Method D 1824–66 and in Saybolt seconds by ASTM Method D 88–56.

(5) Softening point: Softening point shall be determined by ASTM Method E 28–67.

(6) Analytical methods for determining drop-softening point, saponification number, and any other specification not listed under subparagraphs (1) through (5) of this paragraph are available upon request from the Commissioner of Food and Drugs.

[30 F.R. 15845, Dec. 23, 1965, as amended at 31 F.R. 11719, Sept. 1966; 32 F.R. 412, Jan. 14, 1967; 32 F.R. 10508, July 18, 1967; 33 F.R. 7685, May 24, 1968; 34 F.R. 20426, Dec. 31, 1969; 36 F.R. 23291, Dec. 8, 1971]

§ 121.2593 Polyvinylidene fluoride resins.

Polyvinylidene fluoride resins may be safely used as articles or components of articles intended for repeated use in contact with food, in accordance with the following prescribed conditions:

(a) For the purpose of this section, the polyvinylidene fluoride resins consist of basic resins produced by the polymerization of vinylidene fluoride.

(b) The finished food-contact article, when extracted at reflux temperatures for 2 hours with the solvents distilled water, 50 percent (by volume) ethyl alcohol in distilled water, and *n*-heptane, yields total extractives in each extracting solvent not to exceed 0.01 milligram per square inch of food-contact surface tested; and if the finished food-contact article is itself the subject of a regulation in this Subpart F it shall also comply with any specifications and limitations prescribed for it by that regulation. (Note: In testing the finished food-contact article, use a separate test sample for each required extracting solvent.)

(c) In accordance with good manufacturing practice, finished food-contact articles containing the polyvinylidene fluoride resins shall be thoroughly cleansed prior to their first use in contact with food.

§ 121.2594 Odorless light petroleum hydrocarbons.

Odorless light petroleum hydrocarbons may be safely used, as a component of nonfood articles intended for use in contact with food, in accordance with the following prescribed conditions:

(a) The additive is a mixture of liquid hydrocarbons derived from petroleum or synthesized from petroleum gases. The additive is chiefly paraffinic, isoparaffinic, or naphthenic in nature.

(b) The additive meets the following specifications:

(1) Odor is faint and not kerosenic.

(2) Initial boiling point is 300° F. minimum.

(3) Final boiling point is 650° F. maximum.

shall bear the name of the additive, carrageenan.

§ 121.1067 Salts of carrageenan.

The food additive salts of carrageenan may be safely used in food in accordance with the following prescribed conditions:

(a) The food additive consists of carrageenan, meeting the provisions of § 121.1066, modified by increasing the concentration of one of the naturally occurring salts (ammonium, calcium, potassium, or sodium) of carrageenan to the level that it is the dominant salt in the additive.

(b) The food additive is used or intended for use in the amount necessary for an emulsifier, stabilizer, or thickener in foods, except for those standardized foods that do not provide for such use.

(c) To assure safe use of the additive, the label and labeling of the additive shall bear the name of the salt of carrageenan that dominates the mixture by reason of the modification, e.g. "sodium carrageenan," "potassium carrageenan," etc.

§ 121.1068 Furcelleran.

The food additive furcelleran may be safely used in food in accordance with the following prescribed conditions:

(a) The food additive is the refined hydrocolloid prepared by aqueous extraction of furcellaria fastigiata of the class Rodophyceae (red seaweed).

(b) The food additive conforms to the following:

(1) It is a sulfated polysaccharide the dominant hexose units of which are galactose and anhydrogalactose.

(2) Range of sulfate content: 8 percent to 19 percent, on a dry-weight basis.

(c) The food additive is used or intended for use in the amount necessary for an emulsifier, stabilizer, or thickener in foods, except for those standardized foods that do not provide for such use.

(d) To assure safe use of the additive, the label and labeling of the additive shall bear the name of the additive, furcelleran.

§ 121.1069 Salts of furcelleran.

The food additive salts of furcelleran may be safely used in food in accordance with the following prescribed conditions:

(a) The food additive consists of furcelleran, meeting the provisions of § 121.1068, modified by increasing the concentration of one of the naturally occurring salts (ammonium, calcium, potassium, or sodium) of furcelleran to the level that it is the dominant salt in the additive.

(b) The food additive is used or intended for use in the amount necessary for an emulsifier, stabilizer, or thickener in foods, except for those standardized foods that do not provide for such use.

(c) To assure safe use of the additive, the label and labeling of the additive shall bear the name of the salt of furcelleran that dominates the mixture by reason of the modification, e.g. "sodium furcelleran," "potassium furcelleran," etc.

§ 121.1070 Fatty acids.

The food additive fatty acids may be safely used in food and in the manufacture of food components in accordance with the following prescribed conditions:

(a) The food additive consists of one or any mixture of the following straight-chain monobasic carboxylic acids and their associated fatty acids manufactured from fats and oils derived from edible sources: Capric acid, caprylic acid, lauric acid, myristic acid, oleic acid, palmitic acid, and stearic acid.

(b) The food additive meets the following specifications:

(1) Unsaponifiable matter does not exceed 2 percent.

(2) It is free of chick-edema factor:

(i) As evidenced during the bioassay method for determining the chick-edema factor as prescribed in paragraph (c)(2) of this section; or

(ii) As evidenced by the absence of chromatographic peaks with a retention time relative to aldrin (RA) between 10 and 25, using the gas chromatographic-electron capture method prescribed in paragraph (c)(3) of this section. If chromatographic peaks are found with RA values between 10 and 25, the food additive shall meet the requirements of the bioassay method prescribed in paragraph (c)(2) of this section for determining chick-edema factor.

(c) For the purposes of this section:

(1) Unsaponifiable matter shall be determined by the method described in the most recent edition of "Official Methods of Analysis of the Association of Official Agricultural Chemists."

(2) Chick-edema factor shall be determined by the bioassay method described in Official Methods of Analysis of the Association of Official Agricultural Chemists, 10th Edition (1965), sections 26.087 through 26.091.

(3) The gas chromatographic-electron capture method for testing fatty acids for chick-edema shall be the method described in the "Journal of the Association of Official Analytical Chemists," Volume 50 (No. 1), pages 216–218 (1967), or the modified method using a sulfuric acid clean-up procedure, as described in the "Journal of the Association of Official Analytical Chemists," Volume 51 (No. 2), pages 489–490 (1968).

(d) It is used or intended for use as follows:

(1) In foods as a lubricant, binder, and as a defoaming agent in accordance with good manufacturing practice.

(2) As a component in the manufacture of other food-grade additives.

(e) To assure safe use of the additive, the label and labeling of the additive and any premix thereof shall bear, in addition to the other information required by the act, the following:

(1) The common or usual name of the acid or acids contained therein.

(2) The words "food grade," in juxtaposition with and equally as prominent as the name of the acid.

[30 F.R. 15845, Dec. 23, 1965, as amended at 31 F.R. 11215, Aug. 25, 1966; 32 F.R. 11432, Aug. 8, 1967; 33 F.R. 9016, June 19, 1968]

§ 121.1071 Salts of fatty acids.

The food additive salts of fatty acids may be safely used in food and in the manufacture of food components in accordance with the following prescribed conditions:

(a) The additive consists of one or any mixture of two or more of the aluminum, calcium, magnesium, potassium, and sodium salts of the fatty acids conforming to § 121.1070.

(b) The food additive is used or intended for use as a binder, emulsifier, and anticaking agent in food in accordance with good manufacturing practice.

(c) To assure safe use of the additive, the label and labeling of the additive and any premix thereof shall bear, in addition to the other information required by the act, the following:

(1) The common or usual name of the fatty acid salt or salts contained therein.

(2) The words "food grade," in juxtaposition with and equally as prominent as the name of the salt.

§ 121.1072 Hydrogen cyanide.

The food additive hydrogen cyanide may be present as a residue in certain processed foods in accordance with the following prescribed conditions:

(a) The food additive is present as a result of its use as a fumigant.

(b) The residues of hydrogen cyanide shall not exceed the following levels:

(1) 125 parts per million in cereal flours.

(2) 90 parts per million in cereals that are cooked before being eaten.

(3) 50 parts per million in uncooked ham, bacon, and sausage.

(4) 200 parts per million in cocoa.

(c) Where tolerances are established under both sections 408 and 409 of the act on the raw agricultural commodity and on the processed food, respectively, the total residues of hydrogen cyanide in or on the processed food shall not be greater than that designated in paragraph (b) of this section.

(d) To assure safe use of the additive, the label and labeling of the pesticide formulation containing the food additive shall conform to the label and labeling registered by the United States Department of Agriculture.

§ 121.1073 Potassium iodide.

The food additive potassium iodide may be safely used in accordance with the following prescribed conditions:

(a) The food additive is used as a source of iodine in foods for special dietary use, when the food is marketed under labeling which provides that the maximum daily intake of the additive does not exceed 0.15 milligram of iodine.

(b) To assure safe use of the additive, in addition to the other information required by the act, the label of the additive shall bear:

(1) The name of the additive.

(2) A statement of the concentration of the additive in any mixture.

§ 121.1074 Piperonyl butoxide.

The food additive piperonyl butoxide may be safely used in accordance with the following prescribed conditions:

(a) It is used or intended for use in combination with pyrethrins for control of insects:

(1) In cereal grain mills and in storage areas for milled cereal grain products, whereby the amount of piperonyl butoxide is at least equal to but not more than 10 times the amount of pyrethrins in the formulation.

(2) On the outer ply of multiwall paper bags of 50 pounds or more capacity in amounts not exceeding 60 milligrams per

The Fatty Acids of Tall Oil and Their Ethyl and Glyceryl Esters as Fodder Ingredients

III. The Ethyl Esters in the Feeding of Hens[1]

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In previous papers the chemical and physical properties of the fatty acids of tall oil and of their derivatives (Antila *et al.*, 1962) and the use of ethyl esters of the fatty acids of tall oil in the feeding of milk cattle (Antila *et al.*, 1963*a*, 1963*b*) have been treated. The studies in question revealed that ethyl esters of the fatty acids of tall oil can be used as animal fodder in the feeding of dairy cattle and that the use of this kind of additional feed has a favourable effect in particular on the properties of the milk fat.

It can be seen from the literature review compiled by Suhonen and Antila (1962), dealing with the fat content of the fodder in poultry feeding, that the use of small fat quantities is generally considered advantageous as a means of increasing the concentration of the fodder substances. For this reason, comparison tests with animals were undertaken, aiming at clarification of the possibilities of using ethyl ester of the fatty acids of tall oil as supplementary rations for hens.

Material and methods

The ethyl ester of the fatty acids of tall oil was prepared by the method presented by Antila *et al.* (1962) and admixed with the dry

[1] This study was subsidized by a grant from the Finnish Natural Resources Research Foundation.

feed. The basic fodder was the so-called dry egg production feed mixture having the following composition (in per cent):

maize meal feed	25.0
wheat meal feed	17.0
oat meal feed	7.0
barley meal feed	8.5
wheat bran	8.0
herring meal	11.0
coarse soy-bean meal	9.0
coarse linseed meal	
molasses	
sunflower seed meal	
fodder yeast	
grass meal	14.5
vitamin mixture	
ground limestone for feeding	
coarse bone meal	
salt mixture	
	100.0

The vitamin mixture included vitamin A, D_3, B_2, B_{12} and E and pantothenic acid.

The salt mixture contained, in addition to sodium chloride (94.7 %), manganosulphate, iron sulphate, copper sulphate and potassium iodide.

The basic feed contains 20 % crude protein and 3 % crude fat extractable with ether. In addition to the dry feed, the hens were given a grain mixture with one-half oats and one-half barley. The crude protein content of the grain mixture varied between 10 and 12 % and its ether-extractable crude fat, between 3.3 and 3.6 %. The hens were of white Leghorn breed.

In the present investigation the following methods of analysis were used. The fat determination of eggs was made according to AOAC and that of the iodine number according to Hanus (Kaufmann 1958). The lipids were extracted from the egg yolks with ether-petrol ether mixture (1:1). The fats were methylated for the analyses by gas chromatography in the manner presented by Kärkkäinen and Antila (1960). The gas chromatography apparatus used in this work was a Perkin Elmer Model 800 with 2 meters BDS columns. The temperature was raised during the run from 150 to 210°C at the rate of 6.25 degrees per minute.

0174

Arrangement of the tests

Three sets of feeding tests were carried out. The first, second and third feeding test took place during the periods May to July 1962, December 1962 to May 1963 and February to April 1964, respectively.

In all three tests the hens were divided into three groups, each of which was given the same basic feed, namely, the dry egg-production feed mixture. To the first group, No. 1, this feed mixture was administered as such, while to the mixture given to the hens in groups No. 2 and 3 ethyl esters of the fatty acids of tall oil had been added at 5 % and 10 %, respectively. The hens were allowed to eat of the dry feed mixture ad lib. and the consumed quantities were ascertained by weighing. In addition to the dry feed mixture, 60 g of the grain mixture were given per day and hen. All tests were carried out in the poultry house of the School for Poultrymen in Hämeenlinna. The eggs needed for analysis were taken at random from the output of each test group.

Results

The results recorded in the first feeding test have been shown in Table 1. The grain ration, 60 g per day, was consumed completely by the hens, whereas the consumption of dry feed mixture varied in the different groups. It was highest in the control group, while in the other groups the addition of esters impaired the hens' appetite. Because the consumption of dry feed mixture varied in the manner evident from the table, the ester content of the total feed ration was also computed. It was found to have varied between 2.3 and 2.6 % in group No. 2, averaging 2.4 %, and between 4.3 and 4.8 % in group No. 3, with the average of 4.6 %. The smaller addition in group No. 2 seemed to increase the egg production, whereas the higher addition in group No. 3 continuously lowered it.

As the first test seemed to indicate that a small addition of esters of the fatty acids of tall oil has a boosting effect on egg production, another test of longer duration was undertaken. Its results can be seen in Table 2. Fairly equal amounts of the dry feed mixture were consumed by the hens of the control group and of the group in which the smaller ester quantity (on the average 2.7 % of the total feed ration) was administered, but the individuals in the group with higher ester dosage (on the average 4.1 %) ingested about one-third less than the other two

Table 1. *Feeding test, May to July 1962.*

	Month	Average number of hens	Egg production (%)	Dry feed mixture g	Grains g	Total g	Ethyl ester in the total feed ration g	Ethyl ester in the total feed ration %
Group No. 1	IV	27.0	63.5					
(Controls)	V	27.0	64.1	81	60	141		
	VI	25.5	64.4	71	60	131		
	VII	23.8	62.8	69	60	129		
Group No. 2	IV	52.0	61.0					
5 % ethyl ester	V	51.4	54.8	50	60	110	2.5	2.3
in the dry feed	VI	46.7	55.0	54	60	114	2.7	2.4
mixture	VII	45.8	59.9	62	60	122	3.1	2.6
Group No. 3	IV	64.0	59.7					
10 % ethyl ester	V	63.5	55.6	55	60	115	5.5	4.8
in the dry feed	VI	56.0	52.7	45	60	105	4.5	4.3
mixture	VII	52.9	50.6	50	60	110	5.0	4.5

groups. The egg production percentage of all groups shows a decline in January. During February an increase in groups No. 1 and 2 is observed but that of group No. 3 is seen to decrease. Subsequently, the decrease continued in all groups. During the entire test period, the egg production percentage decreased by 5 % in the control group and by 15–16 % in the other two. The test thus revealed that also a smaller ester addition lowers the egg production over longer periods.

Since the feeding may also affect the fertilization of the eggs and the result of hatching, two hatching tests were carried out with eggs derived from the different test groups. Their results are seen in Table 3.

The results of both hatchings are mutually well consistent and the percentages are good. The fertilization and hatching percentage are both slightly better in the control group than in the groups with ester administration.

The purpose of the third feeding test was, in the first place, to corroborate the results relating to the composition of the eggs. In this test the egg production was equal in magnitude as in the preceding tests, as was also the consumption of feeds. The feed ration contained on the average ethyl ester at 2.6 and 4.7 %, respectively. In the control group the highest consumption of dry feed mixture was noted; the consumption was lowest in the group with the higher ester dosage. The results of the test have been compiled in Table 4.

014

TABLE 2. *Feeding test, December 1962 to May 1963.*

	Month	Average number of hens	Egg production (%)	Dry feed mixture g	Grains g	Total g	Ethyl ester in the total feed ration g	Ethyl ester in the total feed ration %
Group No. 1 (Controls)	XII	36.7	72.7	80	60	140		
	I	36.0	68.1	53	60	113		
	II	35.4	73.1	59	60	119		
	III	34.9	68.0	47	60	107		
	IV	33.4	66.4	52	60	112		
	V	29.1	69.0	78	60	138		
Group No. 2 5 % ethyl ester in the dry feed mixture	XII	30.0	85.2	69	60	129	3.5	2.7
	I	30.0	82.7	57	60	117	2.9	2.4
	II	29.4	84.4	63	60	123	3.2	2.6
	III	29.0	75.4	51	60	111	2.6	2.3
	IV	29.0	76.2	82	60	142	4.1	2.9
	V	26.2	71.6	83	60	143	4.2	2.9
Group No. 3 10 % ethyl ester in the dry feed mixture	XII	29.3	71.1	51	60	111	5.1	4.6
	I	26.8	63.3	40	60	100	4.0	4.0
	II	26.0	63.2	30	60	90	3.0	3.3
	III	25.1	63.4	30	60	90	3.0	3.3
	IV	25.0	61.7	50	60	110	5.0	4.5
	V	23.1	60.1	47	60	107	4.7	4.4

TABLE 3. *Fertilization and hatching percentage of eggs.*

Group	Fertilization percentage: Controls	Fertilization percentage: Ethyl ester in the dry feed mixture 5 %	Fertilization percentage: Ethyl ester in the dry feed mixture 10 %	Hatching percentage: Controls	Hatching percentage: Ethyl ester in the dry feed mixture 5 %	Hatching percentage: Ethyl ester in the dry feed mixture 10 %
First hatching	94.6	87.9	89.2	80.0	81.3	76.9
Second hatching	93.2	87.7	90.8	83.8	79.2	79.2
Mean	93.8	87.8	90.0	81.9	80.2	78.0

In order to clarify the composition and properties of the eggs, the weights of shell, egg white and yolk were separately determined for 15 random samples from each feeding group. Furthermore, the fat content of the yolk was determined and the iodine number and fatty acid composition of the isolated lipids were determined. In conditions consistent with actual practice, the flavour of the eggs, their beating quality and the stability of the egg-beat were assessed.

TABLE 4. *Feeding test, February to April 1964.*

	Month	Average number of hens	Egg production (%)	Dry feed mixture g	Grains g	Total g	Ethyl ester in the total feed ration g	Ethyl ester in the total feed ration %
Group No. 1 (Controls)	II	39.0	79.6	77	60	137		
	III	39.0	78.1	90	60	150		
	IV	38.1	68.8	69	60	129		
Group No. 2 5 % ethyl ester in the dry feed mixture	II	42.0	81.2	60	60	120	3.0	2.5
	III	42.0	77.5	70	60	130	3.5	2.7
	IV	41.0	74.7	64	60	124	3.2	2.6
Group No. 3 10 % ethyl ester in the dry feed mixture	II	47.0	77.0	52	60	112	5.2	4.6
	III	47.0	72.8	57	60	117	5.7	4.9
	IV	45.1	70.0	50	60	110	5.0	4.5

TABLE 5. *Effect of the feeding of ethyl esters of the fatty acids of tall oil an the weight of the eggs.*

The figures refer to 15 eggs each.

Ethyl ester in the dry feed (%)	Feeding test No.	Weight of the eggs, g	Shell weight g	Shell weight %	Egg-white weight g	Egg-white weight %	Yolk weight g	Yolk weight %	Handling losses per cent units
0	2	897.7	86.2	9.6	533.4	59.4	266.9	29.7	1.3
	3	914.0	85.3	9.3	537.8	58.8	276.4	30.2	1.7
5	2	889.6	85.2	9.6	521.6	58.6	266.9	30.0	1.8
	3	885.3	89.0	10.5	512.3	58.1	267.4	30.5	0.9
10	2	861.0	85.4	9.9	504.3	58.6	263.7	30.6	0.9
	3	872.7	89.8	10.3	506.9	58.1	268.8	30.8	0.8

Table 5 shows the effect of ethyl ester feeding on the weight of the eggs. It is seen that the 15-egg-weight was clearly reduced by the feeding of ethyl ester. The reduction in weight is primarily due to smaller quantity of the egg-white constituent, since the yolk weights and shell weights were nearly equal in the different groups.

In Table 6 the effect of ethyl ester feeding on the composition of the yolk fat has been presented. According to the results, the fat content of the yolk is only slightly affected by the said kind of feeding, the fat

TABLE 6. *Effect of ethyl ester feeding on the fatty acid composition of the eggs lipids.*

Ethyl ester in the dry feed (%)	Feeding test No.	Fat content %	Iodine number of the fat	Fatty acid composition (%)					
				C_{14}	C_{16}	$C_{16}1=$	C_{18}	$C_{18}1=$	$C_{18}2$
0	2	31.7	77.3	0.8	29.6	2.8	13.9	35.5	17.1
	3		77.5						
5	2	32.5	77.4	0.6	26.3	4.1	10.7	43.4	15.0
	3		78.8						
10	2	32.7	78.4	0.6	25.2	4.8	8.5	47.8	13.3
	3								

percentage having increased by one per cent unit when the ethyl ester dosage was 4.7 %. The iodine number of the fat is also slightly elevated. On the other hand essential changes have taken place in the fatty acid composition. The quantity of oleic acid has greatly increased, namely 7.9 and 12.3 per cent units in the groups in which ethyl ester was administered at 2.6 and 4.7 %, respectively. The quantity of palmitic, stearic and linolic acid has correspondingly gone down as a result of ethyl ester feeding.

On the strength of organoleptic assessment no flavour differences were observed in the eggs of the second feeding test, whereas in the third test the eggs of the test group with the highest ethyl ester dosage had a flavour nuance which could not be more closely defined.

Eggs intended to be used in confectionery are required to have not only faultless flavour but also good beating quality and good stability of the egg-beat. A test elucidating this aspect was undertaken with eggs from the second feeding test in a confectioner's shop. The eggs produced by the hens of the control group were good to beat and the stability of the egg-beat was satisfactory. The eggs of the hens to whom esters had been fed could certainly be beaten, but the egg-beat was not stable.

With the eggs from the third test the beating test was carried out three times in another confectioner's shop. The results were the same every time. The eggs from the control group made the best beat, in about seven minutes, and the pastries rose well. No remarkable differences were noted between the eggs of the two ester feeding groups in respect of beating time, which was about nine minutes. The pastries made with eggs produced by the hens to whom esters had been administered at the higher dosage were poorest in appearance; they did

TABLE 7. *Effect of ethyl ester feeding in the fatty acid composition of hens' carcass fat.*

	Ethyl ester administered at		
	0 %	5 %	10 %
Iodine number of the fat . . .	84.2	83.3	85.6
C_{14}	0.4	0.4	0.5
C_{16}	19.1	20.6	16.9
$C_{16}1=$	2.9	3.3	2.7
C_{18}	5.8	5.5	5.1
$C_{18}1=$	46.9	47.8	46.0
$C_{18}2=$	24.8	21.1	26.5
$C_{18}3=$	0.7	0.5	0.9
C_{20}	1.0	0.7	0.8

not rise and their surface was uneven. The same defects, though in slighter degree, were noted when eggs from the hens fed with ethyl ester at 2.6 % were used. Consequently, it would seem that the feeding of ethyl esters of the fatty acids of tall oil to hens is not recommendable in view of baking.

The effect exerted by feeding of ethyl esters of the fatty acids of tall oil to hens on their carcass fat was clarified by determining the fatty acid composition of this fat. From the results shown in Table 7 the inference can be drawn that at least in the present instances the ethyl ester administration had no distinct influence on the fatty acid composition of the carcass fat. It is a notable fact that oleic and linolic acid contribute with a very high percentage, both together about 70 %, to the said fats.

On judging the present results, the arrangement of the tests has to be taken into account, namely, that in the test groups the ethyl ester was administered admixed to the normal feed of the control group. The fat content of the feed has thus been higher in the test groups proper than in the control group and this is thought to be partly accountable for the values revealed by the present results.

Summary of results

In the present study the suitability of ethyl esters of the fatty acids of tall oil for use in the feeding of hens was clarified with the aid of three long-term feeding tests. It was found that when such ethyl ester was added to the basic

feed which was administered to the control group as such, the egg production of the hens decreased but fertilization of the eggs and hatching results were entirely normal. The baking characteristics of the eggs were distinctly impaired, however. The fat content of the yolk and the iodine number of the fat were not significantly changed. Considerable changes took place in the fatty acid composition of the yolk fat. The quantity of oleic acid increased strongly, with simultaneous decrease of the palmitic, stearic and linolic acid quantities. Ethyl ester feeding caused a reduction of the egg weight, which was due to reduction of the egg-white constituent in the first place. Ethyl ester feeding was not observed to have exerted any influence on the fatty acid composition of the carcass fat.

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The Fatty Acids of Tall Oil and Their Ethyl and Glyceryl Esters as Fodder Ingredients

II. The ethyl esters in the feeding of milk cows

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The fatty acids of tall oil and the use of their derivatives as fodder ingredients were discussed in an earlier publication (Antila *et al.*, 1962). It was concluded that especially the ethyl esters of the acids are economically advantageous under Finnish conditions in view of the availability of raw materials and ease of preparation. The composition of Finnish butter fat varies in different seasons of the year and contains relatively low levels of unsaturated fatty acids especially during the indoor feeding period in Eastern Finland (Antila, 1962). As a consequence the production of butter of the right consistency is difficult at times. Since butter is the major product of the dairy industry in Finland, a variable consistency is both a commercial and an economic problem which it has not been possible to solve by modifying the machinery, processes or the cattle fodder.

The incorporation of fats in the diet of cattle has been discussed widely as is evident from the review of Suhonen and Antila (1961). The value of ethyl esters of tall oil fatty acids in cattle fodder has not apparently been investigated, however.

The aim of this study was to investigate the stability of ethyl esters of tall oil fatty acids in fodder and the effect on the composition of milk fat of feeding them to cows.

Material and Methods

The ethyl esters of tall oil fatty acids were prepared by the method of Antila *et al.* (1962). The ester mixture was then combined with various

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powdered materials. One was a powder made from whey produced in the manufacture of Emmenthal cheese and one a drum-dried skimmed milk powder. The third was a powder prepared by drying and grinding clover (dried grass meal). The fourth material was roasted soybean meal.

The following analytical methods were employed: milk fat according to Gerber (Davies, 1959); milk proteins according to Kjeldahl (Ling, 1956); iodine value according to Hanus (Kaufmann, 1958); conjugated and non-conjugated diethenoid acids by the A.O.C.S. Tentative Method L 12 a, 55; peroxide value according to Lea (Kaufmann, 1958); and Swift's test (Mehlenbacher, 1945).

Experimental Procedures

In the stability studies, the ester mixture was mixed with the various fodder components in different ratios and the resulting mixtures were stored in the dark at room temperature for eight weeks. The oxidation of the ester mixture and its mixtures with the other components was followed by determining peroxide values.

Two feeding experiments were arranged, both during the indoor feeding period. In each experiment, there were four cows in different stages of lactation period in the test and the control groups. The experiments extended over a preliminary period of 20–25 days, a transition period of 5 days, an experimental period of 25–30 days, a transition period of 5 days and a final period of 5 days. The amount of fodder given to the cows varied with the milk production and live weight.

In the first experiment, each cow received (in kg):

Hay	5–6
Marrow kale silage	15
Oatmeal feed	2–3.5
Soya bean meal (coarse)	0.2–0.5

After the preliminary period 4 per cent of the ethyl esters of the tall oil fatty acids was added to the concentrates of the test group. Both groups of cows received the same fodder in the final period as in the preliminary period.

The basic fodder received by all cows in the second experiment consisted (in kg) of:

Hay	5.0
AIV silage (grass)	15–20
Dried molasses beet pulp	1.0
Molasses	0.5
Concentrate mixture	0.5–1.0

This concentrate mixture comprised (%)

Barley meal feed	15
Wheat meal feed	15
Soya bean meal (coarse)	15
Linseed oil cake	13
Maizena	10
"Herutushera" (meat and bone meal + whey powder)	10
Dried grass meal	10
Phosphorus yeast	6
Disodium phosphate	5
"DEB Vitan"	1

and as test fodder the concentrate

Mixture II	3.5 5.0

This test fodder was composed of (%)

Oatmeal feed	40
Barley meal feed	40
Skimmed milk powder	10
Whey powder	10

All the cows were fed the test fodder II, but the test fodder the test cows received during the first transition period and the experimental period contained 3 per cent of ethyl esters of tall oil fatty acids.

The yield of milk, milk fat and milk protein was followed throughout the experimental period.

For the examination of the milk fat, it was separated from the milk by centrifuging and churning, the butter granules were melted and the fat filtered through dry filter paper.

In the oxidation tests, both the clear butter fat obtained in this way and an emulsion of the washed butter granules were examined.

Results

The ethyl esters of tall oil fatty acids are relatively prone to oxidation as they do not contain natural anti-oxidants to any extent. The rate of oxidation of the ester mixture as such and when present in fodder with and without added dried grass meal is shown in Fig. 1. The more rapid oxidation of the esters in the meal feed than as an oil is probably due to the larger interface between air and the oil adsorbed on the porous meal feed. When 4 % dried grass meal is present in the ester mixture, the peroxide value remains at a low level during storage owing to the anti-oxidants in the dried grass meal. The stabilising effect of the

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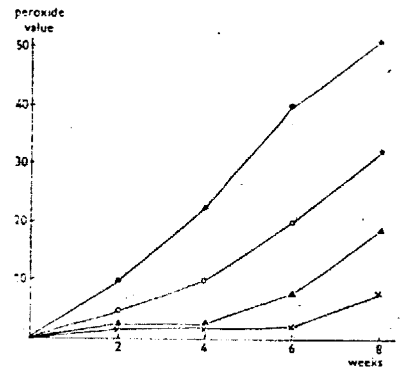

Fig. 1. The oxidation of the ethyl esters of tall oil fatty acids at room temperature. As meal feed roasted soybean meal. ○ Ethyl esters. ◉ Ethyl esters (10 %) + roasted soybean meal (90 %). × Ethyl esters + dried grass meal (4 %). ▲ The mixture of ethyl esters and dried grass meal (10 %) in meal feed.

dried grass meal becomes evident also when its mixture with the ethyl esters is incorporated in the meal feed.

The oxidation of the ester mixture when admixed with skimmed milk powder and whey powder is relatively slow owing to the reducing agents present in these powders. As judged by the peroxide values, the best storage properties were those of the mixture of ethyl esters, dried grass meal and whey powders (Fig. 2).

Only minor changes in milk yield were observed during the feeding experiments (Fig. 3); the differences were not statistically significant.

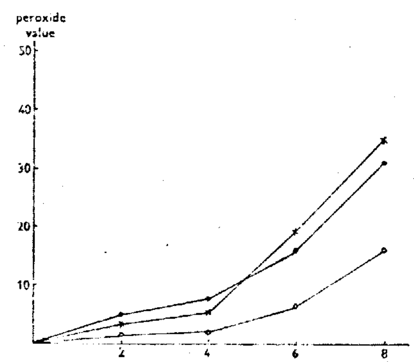

Fig. 2. The oxidation of the ethyl esters in skimmed milk powder and whey powder at room temperature. ○ The mixture of ethyl esters and dried grass meal (10 %) in whey powder. × Ethyl esters (10 %) + whey powder (90 %). ◉ Ethyl esters (10 %) + skimmed milk powder (90 %).

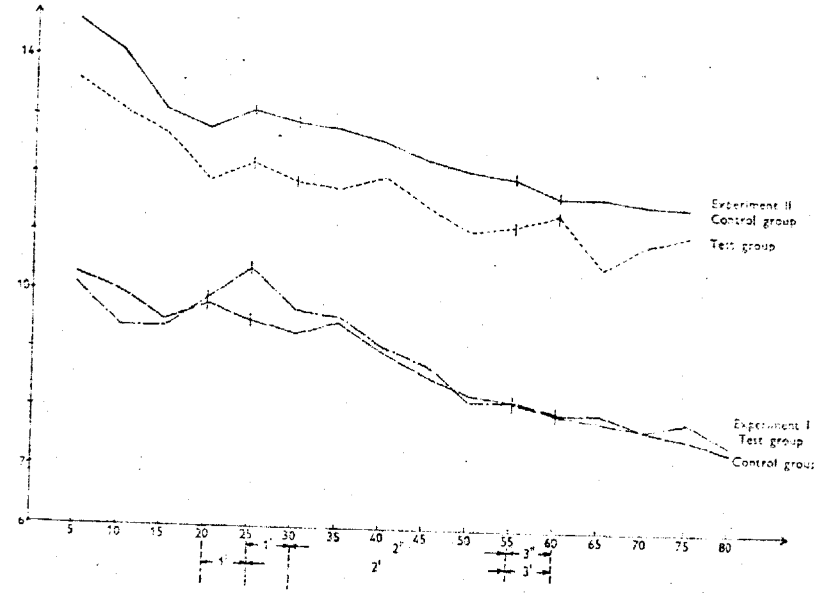

Fig. 3. Variation in milk production during the experiments. 1 = Transition period. 2 = Test period. 3 = Transition period.

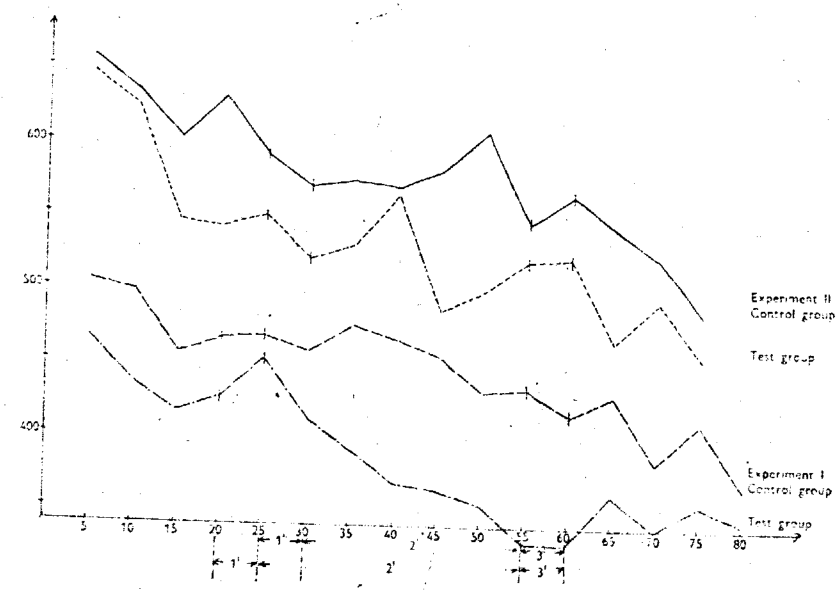

Fig. 4. Variation in milk fat production during the experiments. 1 = Transition period. 2 = Test period. 3 = Transition period.

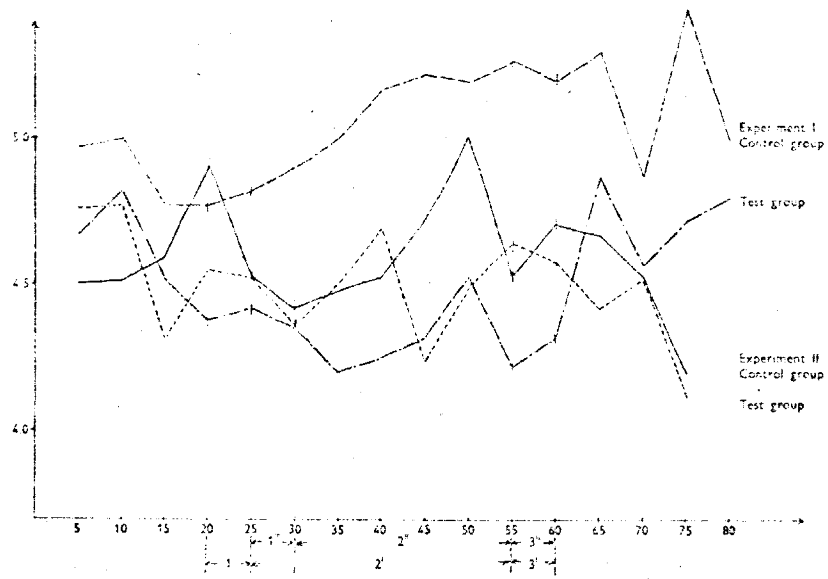

Fig. 5. Variation in fat content of milk during the experiments. 1 = Transition period. 2 = Test period. 3 = Transition period.

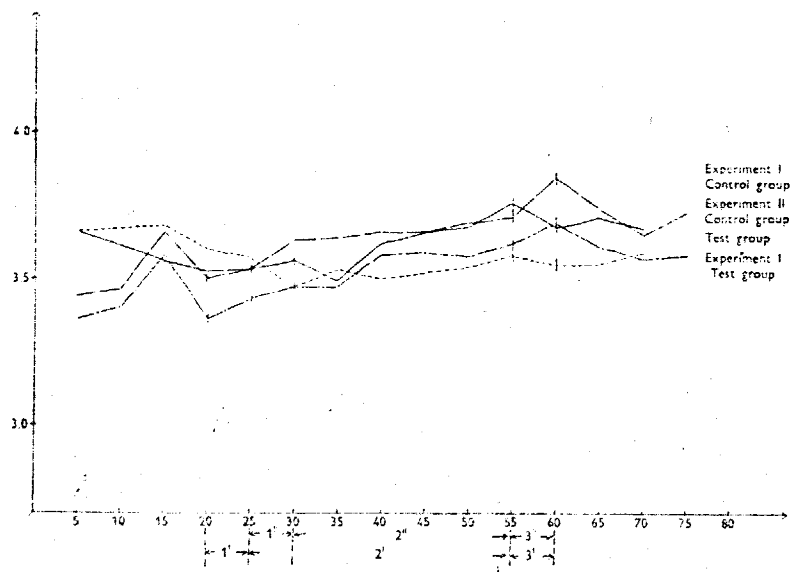

Fig. 6. Variation in protein content of milk during the experiments. 1 = Transition period. 2 = Test period. 3 = Transition period.

TABLE 1. *Changes in the iodine value of milk fat produced by feeding ethyl esters of tall oil fatty acids to cows.*

	Feeding experiment I		Feeding experiment II	
	Test group	Control group	Test group	Control group
Preliminary period	31.9	30.4	30.2	31.3
Test period	38.1	31.4	33.0	31.8
Follow-up period	33.5	31.3	31.8	33.8

The milk fat yield was significantly lower ($P < 0.01$) in the group receiving the ethyl esters than in the control group in the first experiment (Fig. 4), but not in the second experiment. The significant difference in the first experiment is probably a consequence of the higher intake of ethyl esters, which was from 24.7 to 39.0 per cent of the milk fat produced by the cows in question. In the second feeding experiment, the amount of ethyl ester mixture added was 3 per cent of the weight of the fodder and was only 20.3–27.2 per cent of the milk fat produced by the cows which received it. In addition, there were more sugars in the fodder in the second than in the first experiment and this may also have had an effect on the results. The daily variations in the fat content of the milk were fairly large (Fig. 5) and may also have obscured the changes in the milk fat production in the experiments.

The variations in milk protein contents shown in Fig. 6 do not reveal any significant changes as a result of the feeding of the ethyl esters.

TABLE 2. *The effect of ethyl esters of tall oil fatty acids in fodder on the content of diethenoids in milk fat.*

	Feeding experiment I				Feeding experiment II			
	Test group		Control group		Test group		Control group	
	% conjugated	% non-conjugated	% conjugated	% non-conjugated	% conjugated	% non-conjugated	% conjugated	% non-conjugated
Preliminary period	0.63	1.03	0.57	1.04	0.57	1.22	0.58	1.21
Test period	1.02	0.98	0.59	0.98	0.62	1.07	0.55	1.18
Follow-up period	0.65	0.99	0.58	0.88	0.58	1.14	0.64	1.01

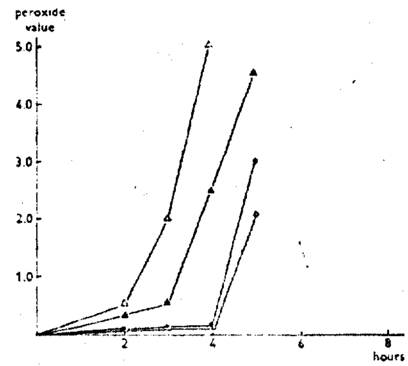

Fig. 7. Oxidation tendency of butter fat and a butter emulsion before and during the feeding of tall oil fatty acid ethyl esters. Temperature 65°C. Rate of oxygen flow 10 litres/hour. ● Butter fat before the feeding (IV 33.6). ○ Butter fat during the feeding (IV 37.8). ▲ Butter emulsion before the feeding. △ Butter emulsion during the feeding.

The data in Table 1 show that the iodine value of the milk fat increased significantly ($P<0.01$) in both experiments as a result of the feeding of the ethyl esters.

The increase in the iodine value is so high that on the iodine value level in question it has an effect on the properties of milk fat from the point of view of butter making. On the basis of the investigations by Peltola & Huumonen (1957), for example, it can be concluded that the difference of 1.6 iodine value units is distinctly manifested in the cutting resistance of Finnish butter.

The changes in the amount of the diethenoids of butter fat as a consequence of the ethyl ester feeding are presented in Table 2.

As seen in Table 2, the proportion of conjugated diethenoids underwent a marked increase in the first experiment. The difference between the test and control groups is statistically significant ($P<0.01$). The proportion did not increase significantly in the second experiment.

Work carried out by Antila *et al.* (1963) on the fatty acid composition of milk fat has revealed that the feeding of ethyl esters of tall oil fatty acids leads to an increase in the oleic acid content and to a decrease in the palmitic acid content. The observed increase in iodine value is probably connected with this and not with any change in the diethenoid content.

Samples of milk fat collected during the first feeding experiment were stored after drying in test tubes in a refrigerator for six months. Peroxide value determinations on these samples revealed no significant differences between the test and control groups. The peroxide values for both groups of samples were of the order of 1.0, and the oxidation of the samples can still be followed by peroxide value determinations.

An increase in the iodine value of butter fat implies a greater tendency to undergo oxidation as shown by Fig. 7. The susceptibility to oxidation was greater for the butter emulsion than for the butter fat.

Summary

The use of ethyl esters of tall oil fatty acids in feeding milk cows has been investigated. The tendency of the ethyl esters to undergo oxidation is relatively great as such and when present in the fodder, but the oxidation can be retarded by adding dried grass meal to the esters before mixing the latter with the other fodder components. Also the oxidation of the esters in admixture with skimmed milk powder and whey powder was followed.

The influence of the ethyl esters on the milk production, milk fat production, protein content of milk and the properties of the milk fat was examined in two feeding experiments. The changes in iodine value and the content of diethenoid fatty acids in butter fat were followed and observations were made on the susceptibility of the butter fat to oxidation. The inclusion of ethyl esters of tall oil fatty acids in cattle fodder leads to an increase in the iodine value of the milk fat produced. If the amounts given are small, they increase the iodine value without lowering the yield of milk fat and also increase the proportion of conjugated diethenoids significantly. The tendency of the butter fat to undergo oxidation is increased when the ethyl esters are added to cattle fodder.

Acknowledgements. The writers express their gratitude to Dr. Veikko Reinikainen, General Manager of the Raisio Factories Ltd., Agronomist O. A. Rauhamaa, Managing Director of the Jokioisten Kartanot (State Farm), Agronomist Mikko Ihamuotila, Manager of Malminkartano (University Experimental Farm), and Prof. Erkki Peltola, Director of the State Institute for Dairy Research, for their cooperation in making this investigation possible.

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Joint FAO/WHO Food Standards Programme
CODEX ALIMENTARIUS COMMISSION

CAC/RS 19-1969

RECOMMENDED INTERNATIONAL GENERAL STANDARD FOR EDIBLE FATS AND OILS

NOT COVERED BY INDIVIDUAL CODEX STANDARDS

FOOD AND AGRICULTURE ORGANIZATION
OF THE UNITED NATIONS
WORLD HEALTH ORGANIZATION

4.2 Flavours

Natural flavours and their identical synthetic equivalents, except those which are known to represent a toxic hazard, and other synthetic flavours approved by the Codex Alimentarius Commission are permitted for the purpose of restoring natural flavour lost in processing or for the purpose of standardizing flavour, as long as the added flavour does not deceive or mislead the consumer by concealing damage or inferiority or by making the product appear to be of greater than actual value. (*)

4.3 Emulsifiers

The following are permitted but only in fats and oils not specifically designated with the name of the plant or animal from which they originate:

	Maximum level of use
4.3.1 Mono- and diglycerides of fatty acids	Not limited
4.3.2 Mono- and diglycerides of fatty acids esterified with the following acids: acetic, acetyltartaric, citric, lactic, tartaric and their sodium and calcium salts	20 g/kg of the emulsifiers specified under 4.3.2 to 4.3.11 inclusive, individually or in combination
4.3.3 Lecithins and components of commercial lecithin	
4.3.4 Polyglycerol esters of fatty acids	
4.3.5 Esters of fatty acids with polyalcohols other than glycerol: Sorbitan monopalmitate, Sorbitan monostearate, Sorbitan tristearate	

(*) Temporarily endorsed.

	Maximum level of use
4.3 Emulsifiers (Cont.)	
4.3.6 1,2 propylene glycol esters of fatty acids	)
4.3.7 Sucrose esters of fatty acids (including sucroglycerides) (*)	)
4.3.8 Stearoyl lactylic acid and its calcium salt (*)	)
4.3.9 Polyglycerol esters of interesterified ricinoleic acid (*)	)
4.3.10 Polyoxyethylene (20) sorbitan monostearate	)
4.3.11 Polyoxyethylene (20) sorbitan monoöleate	)
4.4 Antioxidants	
4.4.1 Propyl, octyl, and dodecyl gallates	100 mg/kg individually or in combination
4.4.2 Butylated hydroxytoluene (BHT) Butylated hydroxyanisole (BHA)	200 mg/kg individually or in combination
4.4.3 Any combination of gallates with BHA or BHT, or both	200 mg/kg, but gallates not to exceed 100 mg/kg
4.4.4 Natural and synthetic tocopherols	Not limited
4.4.5 Ascorbyl palmitate)	200 mg/kg individually or in combination
4.4.6 Ascorbyl stearate)	
4.4.7 Dilauryl thiodipropionate	200 mg/kg

(*) Temporarily endorsed.

Food Protection Committee, Food
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National Academy of Sciences,
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DEFOAMERS

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THE problem of foam control during the production of bakers' yeast has been serious since Marquardt, following the teachings of Pasteur, introduced aeration into the process in 1879. The problem was intensified by the introduction of the Hayduck process using molasses and ammonia as nutrients in 1915. The shortage of grains at that time accelerated the changeover to the Hayduck process. Frey (*4*), and Frey, Kirby, and Schultz (*5*) described these developments in detail. The growth of microorganisms, especially yeast, is inevitably tied to the use of aeration because of the larger yields received. The exact mechanism whereby yields are increased has not been fully elucidated, but de Becze and Liebmann (*1*) in their all-encompassing review on aeration have listed most of the current theories. Gee and Gerhardt (*6*) have also attempted to throw light on this mechanism.

Two general solutions to the foaming problem have been advanced—the use of mechanical foam breakers, such as are employed in the Waldhof fermenter, and the use of additives which destroy or control the foam. Ross (*10, 11*), and Ross and McBain (*12*) have classified and illustrated the substances in common usage as additive defoamers. Equipment has been designed so that defoamer addition is automatic as required. Stefaniak, Gailey, Brown, and Johnson (*14*) and Bilford, Scalf, Stark, and Kolachov (*2*) have described such equipment.

Generally, additive defoamers rely upon the alteration in surface relationships, and Ross (*11*) has explained some of the factors involved. In the use of chemical additives as defoamers other important characteristics deserve consideration. In yeast growth, yields may be seriously impaired, and in antibiotics manufacture, unit yields may be lowered. Goldshmidt and Koffler (*7*) have made a study of the effect of defoamers upon penicillin yield and have found that with the use of lard oils the yield of penicillin is increased significantly. They explained the enhanced yield as a result of the effect upon surface relationships of the organism itself, and show that it is not due simply to the mechanical destruction of foam.

EXPERIMENTAL

A well-known and accepted technique for testing defoamers for use during yeast manufacture is not available. Goldshmidt and Koffler (*7*) have described a method for testing defoamers for use in penicillin manufacture and Sinsheimer (*13*) has described a method for testing foam stability.

The technique described herein is advanced as one suitable for use in testing yeast defoamers. The apparatus consists essentially of a device for passing cleaned, measured air through a mixture of yeast, molasses, and test defoamer at a controlled temperature; meanwhile, the time for defoamer failure is observed. The apparatus is shown in Figure 1. Construction details are shown in Figures 2 and 3.

TEST PROCEDURE. With the foam column in place, the bath is brought to 30° C.

Two grams of active dried yeast are added to 200 ml. of test molasses and the mixture is stirred mechanically at medium speed for 5 minutes.

Molasses is prepared by diluting dark table molasses to a specific gravity of 1.0390 (20°/20°) after adjustment to pH 4.5. Surplus molasses may be preserved by processing in half-pint home canning jars at 100° C. for 25 minutes, followed by an air cool. This arrangement permits enough for one test per jar.

Air flow is adjusted so that after adding the test mixture, the air rate is 0.125 cubic foot per minute. Air must be started before addition of test mixture.

Foam forms immediately and starts to climb in the tower. When tower is half filled, 0.05 ml. of test defoamer is added. Defoamer should be near at hand when starting test, because foam rise in the tower is rapid.

The time is observed and failure is considered to be the point at which foam fills the tower. The failure time is noted.

At the conclusion of an 8-hour test run, yeast is separated from the spent molasses. The filtering device shown in Figure 4 was found to facilitate this operation.

After three washes with cold distilled water, odor and toxicity observations are made.

PREPARATION OF FILTERING DEVICE. To a high-speed wetted filter paper under suction on a Büchner funnel, a thick slurry of high-speed filter aid is added. Sufficient filter aid is used to give a pad about 0.5 inch thick.

Suction is continued and when the filter aid pad is formed, but before it is dry, another filter paper is placed upon it. Water to a depth of about 1 inch is immediately added, which helps weld the "sandwich" into a unit.

In his compilation of defoamers Ross (*10, 11*) has listed fatty acids as having value in controlling foam during yeast production. Tall oil seemed to be an unexplored source of fatty acids for such an application. Dunlap, Hassel, and Maxwell (*3*) and

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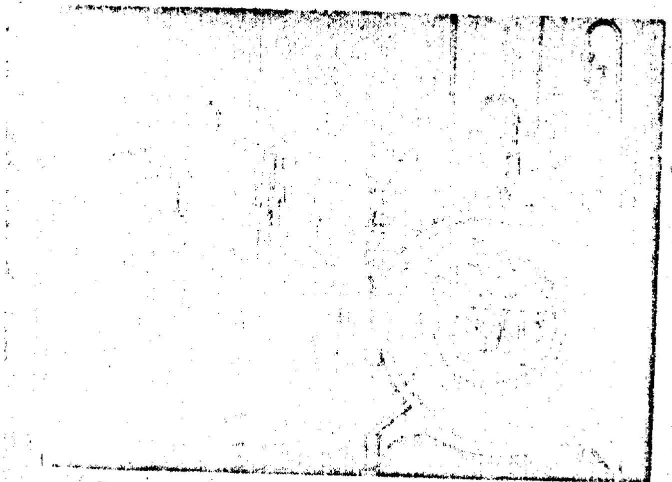

Figure 1. Yeast Defoamer Test Apparatus

·eynolds (9) describe tall oil as being variable but having a gen·ral composition of:

Resin acids, %	40–45
Fatty acids, %	34–45
Sterols, %	3–4
Hydrocarbons	Remainder

·atty acid composition is as follows:

Saturated (mostly palmitic), %	7
Oleic, %	45
Linoleic, %	48
(Conjugated linoleic)	(11)

Because of the availability of reactive hydrogen groups, tall oil ·capable of reacting with ethylene oxide (EO) as described by ·cClellan (8). By reacting tall oil with increasing various molar ·tios of ethylene oxide it is possible to achieve products of in·easing water solubility due to change in hydrophil-hydrophobe ·lance. Accordingly a number of tall oil–ethylene oxide con·nsation products were prepared and tested as shown in Table I. ·olecular weight of refined tall oil used in these studies was as·med to be 300, crude tall oil 315, and ethylene oxide 44. For ·mparison, results with some other test defoamers are shown in ·able II.

Table I. Tall Oil–Ethylene Oxide Condensation Products as Defoamers

Molar Ratio: Tall oil	Molar Ratio: Ethylene oxide	Water Solubility	Foam Control Time, Hours
1	0.00	Floating film	2.5
1	0.25	Slight dispersion	0.5
1	0.50		0.5
1	0.75		6.0
1	1.00		Max.[a]
1	2.00		Max.
1	3.08		Max.
1	5.00		Max.
1	6.00	Increased dispersion	15.0
1	7.00		0.3
1	8.00		0.1
1	12.00	Soluble	Foam enhanced

[a] Maximum, 20 hours.

Table II. Defoamers for Aerated Yeast–Molasses Mixtures

Compound	Foam Control Time, Hours
Loralkyl acid phosphate–1.61 parts ethylene oxide	Foam enhanced
Hexyl acid phosphate–1.40 parts ethylene oxide	0.1
Ethyl acid phosphate–2.40 parts ethylene oxide	Foam enhanced
85% orthophosphoric acid–8.60 parts ethylene oxide	Foam enhanced
Tetradecanol	Maximum[a]
n-Octanol	2.0
D.C. Antifoam A	Maximum
Tridecyl alcohol–3.0 parts ethylene oxide	0.1
Tridecyl alcohol–4.0 parts ethylene oxide	0.1
[illegible] parts ethylene oxide	0.1

[a] Maximum, 20 hours.

A rough screening program which had some merit was employed in the tall oil–ethylene oxide series. Four drops of test defoamer were added to 100 ml. of cold distilled water, and thoroughly mixed. Those defoamers which went into solution immediately always failed, those which remained as a film upon the water were effective, and those showing very slight solution were most effective. These data are shown in Table I.

DISCUSSION

The defoamer testing technique was found to be simple and reproducible. In the early phase of the development of the defoamer test, yeast was added to dilute molasses and poured directly into the foam tower. However, it was observed that in using this technique the nature of the foam changed rather markedly at about 20 minutes operating time. Mechanical premixing was found to eliminate this foam change.

As expected, some variation was encountered in various lots of molasses. To obviate this variable a sufficiently large batch was prepared to supply a complete series of tests. The application of materials closely approximating commercial operation is considered preferable to use of known compositions at variance with actual application. By preparing a large, homogeneous batch of molasses a standard value may be ascertained by use of a known defoamer, such as tetradecanol. Sufficient yeast should be secured for testing the entire batch of molasses and after thorough mixing the surplus should be stored under refrigeration.

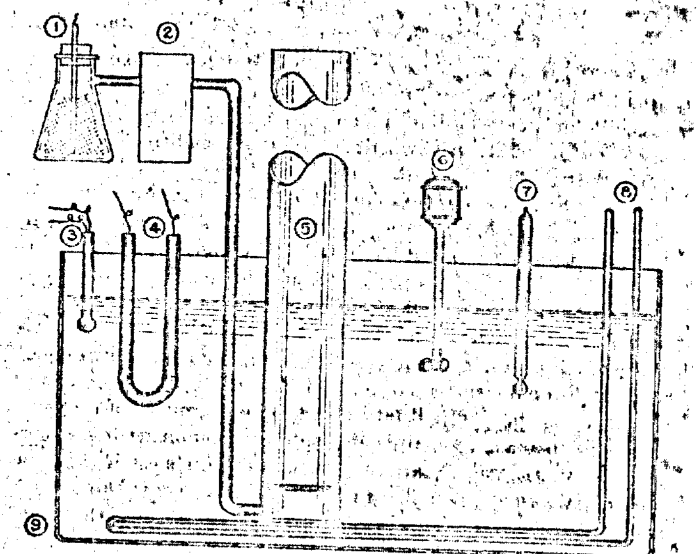

Figure 2. Details of Yeast Defoamer Test Apparatus

1. Air filter. Two-liter sidearm flasks filled with glass wool
2. Orifice flow meter (Ace Glass Co.)
3. Thermoregulator (H-B Instrument Co.)
4. 250-watt heating coil
5. Yeast propagator. Borosilicate glass tubing with 48-mm. inside diameter, 55 cm. high, coarse porosity disk
6. Stirrer
7. Thermometer
8. Cooling coil, 1/4-inch copper tubing
9. Borosilicate glass bath

It was found advisable to start the airflow in the foam tower before adding the test material, otherwise seepage through the fritted disk occurred, which gave a variable test volume and variable air flow. The maximum time employed was 20 hours; this is in excess of the time cycle normally employed during yeast production. Most failures occurred within the first hour.

The filtering technique was found to be fast. It is convenient, and the separated yeast can be readily lifted from the "sandwich" for toxicity observations. If the test was terminated during the growth phase of the yeast, filtration time was always prolonged, probably due to the plugging of the filter pores by the smaller daughter cells, and new issue. Toxic defoamers produced protracted filtration time, because of filter blocking by cellular debris and cell contents. Yeast growth data and microscopic observation are suggested for confirmation of toxicity tests.

In all tests employing tall oil–ethylene oxide condensation products no toxic effects were observed, either microscopically or by filtration performance. Further, no off-odor could be detected after washing.

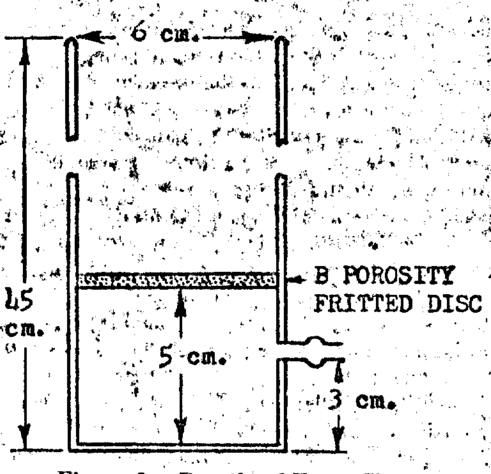

Figure 3. Details of Foam Tower

Ross (*11*) has stated that "the most complete defoamers are usually, but not always, insoluble. In some cases excess of an insoluble antifoam nullifies its effect; hence, in these cases there is an optimum amount where solubility is slightly exceeded." With the approach presented in these studies a handy mechanism is at hand for altering solubility of the defoaming agent. It would appear from the data presented in Table I that complete insolubility is not as desirable in a defoamer as a partial orientation toward the aqueous phase. Thus, untreated tall oil is not as effective as that reacted with 0.7 mole of ethylene oxide, and 1 to 5 moles of ethylene oxide per mole of tall oil are even more effective. Further, with increasing solubility due to enlargement of the hydrophilic portion of the molecule, a lessening in defoamer efficiency is encountered until at a level of 12 moles of ethylene oxide foam enhancement is encountered. Summarizing, a correlation is apparent, starting with fair effectiveness in the low tall oil–ethylene oxide molar ratios, showing a peak in the range 1 to 1 to 1 to 5 and falling off as the hydrophil portion of the molecule lengthens.

Apparently a hydrophil-hydrophobe balance within the defoamer is necessary. Reference to Table II shows that compounds consisting of two hydrophilic portions such as sucrose-ethylene oxide and orthophosphoric acid–ethylene oxide are completely ineffective as defoamers and actually enhance foaming.

As a result of these studies the tall oil–ethylene oxide condensation product of a 1-to-1 mole ratio was selected as the most likely for plant test. Tests in yeast manufacturing plants have shown this compound to be extremely effective in its intended application. A substantiation of the experimental results has thus been achieved.

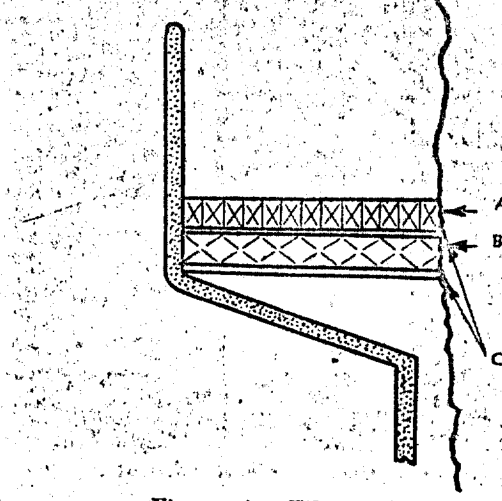

Figure 4. Filter "Sandwich"

A. Yeast
B. High speed filter aid
C. High speed filter paper

ACKNOWLEDGMENT

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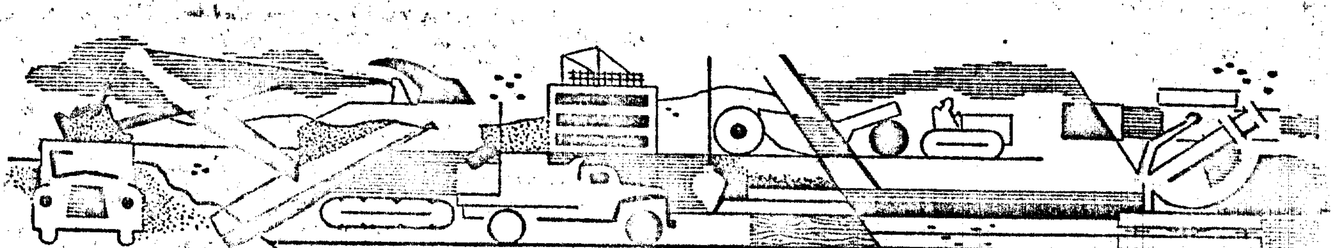

Oak Ridge National Laboratory. 1973

Oleic Acid
<u>The Mutagenicity and Teratogenicity of a Selected Number of Food Additives</u>

(EMIC/GRAS Literature Review), Oak Ridge National Laboratory, Oak Ridge, Tennessee

Page 119

Italian Translation

Tall Oil Fatty Acids and Their Use in The Nutritional Field

Felice Paolini

The extent of fraud practiced in the field of edible vegetable oils is steadily increasing and by this time the chemical industry is in the process of preparing vegetable oils starting from a wide variety of raw materials. In fact, it has been recently established that fatty acids obtained from tall oil are being used for the production of esterified oils, which can be directly used as such for consumption purposes or mixed with other seed oils or olive oil.

Tall oil (Swedish tall pine) is a byproduct obtained in the production of sulfate cellulose from pine wood; its production increased greatly during and after World War II, especially in the U. S. A., in order to satisfy the ever increasing need for drying oils and therefore of unsaturated fatty acids required in the manufacture of alkyd resins, paints, varnishes, soaps, etc.

In the preparation of sulfate cellulose a black and syrupy liquid is formed ("black liquor soap"), from which, under the action of sulfuric acid, washing with water and decanting, crude tall oil is obtained, consisting of a mixture of fatty acids, resin acids unsaponifiable products. The percentage of these components varies according to the type of wood used and the region where it originates. However, industry is able to reduce such variations to a minimum by using suitable mixtures and thus supplying commercial tall oils with a 2-3% content of fatty and resin acids and a maximum content of 1% unsaponifiables.

In view of the fact that individual components of crude tall oil have an increasingly greater field of application, the chemical industry has been able to produce various types of distilled tall oils (with a different percentage of fatty and resin acids) by subjecting crude tall-oil to fractional distillation, in order to obtain fatty acids almost free of resin acids (1%) and resin acids almost free of fatty acids (1%).

According to Hezel (1), crude tall oil consists of 30-50% resin acids, 60-45% fatty acid, 1-6% oxidized fatty and resin acids, and 1-10% unsaponifiables.

Tall oil fatty acids consist primarily of oleic and linoleic acids with a small amount of saturated fatty acids, such as palmitic and lignocerinic acid. Among the unsaturated acids, H. Niessen (2) has identified with certainty also the presence of linolenic acid, while Browning and Calkin (3) have recorded ricinoleic and brassidic acids among the unsaturated acids, and palmitic, lauric, myristic and lignocerinic acids among the saturated acids.

Tall oil resin acids are similar to those found in rosin, and F. J. Ball and W. J. Vardell (4) have shown, by spectrophotometric analysis, that about half of the resin acids consist of abietic and neoabietic acids. In addition, other resin acids are present, which differ from abietic acid in the number or location of double bonds or in the structure of the chain, such as dehydroabietic acid, tetrahydroabietic acid, d-pimaric acid, and iso-d-pimaric acid.

The unsaponifiable content of tall oil consists mainly of a mixture of hydrocarbons, long-chain alcohols and sterols. Present among the hydrocarbons are abietene, diterpenes, terpene polymers and polymers of decarboxylated resin acids; among the alcohols, lignocerylic alcohol is found, and among the sterols beta-sitosterol.

Naturally, during distillation of tall oil, unsaponifiable products are distilled according to their boiling points. Abietene goes over with the head fractions, while sterols and other alcohols with higher boiling points remain in the tall oil pitch. Therefore, distilled tall oil and tall oil fatty acids are free of sterols and high-boiling alcohols. Jacini(5), in a study on the use of tall oil and the isolation of certain sterols, states that there is no doubt about the identity of tall oil phytosterols with those recovered from other plants (especially, soybeans); in another study (6) on an Italian tall oil pitch, Jacini did not find lignoceric alcohol, contrary to the statement made by Sandquist, but rather normal arachic alcohol.

Tall oil pitch, rich in sterols, has thus become an important raw material for the production of beta-sitosterol, used in the synthesis of sex hormones and other biologically active compounds. Again Jacini (7) has described a practical method for the extraction of sterols from tall oil "sulfate soap" and the successive oxidation to dehydroandrosterone of the technical mixture, obtained from the sterols, not subjected to special purificatioh and fractionation.

It is evident from the above that the importance of tall oil is greatly increased by the fact that it affords the possibility of utilizing sterols of plant origin, instead of cholesterol, in the preparation of certain hormones, starting from low-cost raw materials.

Thus, industrial applications of crude tall oil and its derivatives are quite numerous today, and it is certain that the chemical industry will be able to expand, as time goes by, their already vast field of application.

On the other hand, it was not conceivable, until two or three years ago, that industry would be able to utilize these products also in the field of nutrition. Unfortunately, today such an utilization has become a bitter reality, and the incentive for such an illicit use is the considerably lower cost of these products, compared to the cost of natural vegetable oils.

In order to obtain a better knowledge of the composition of tall oil derivatives and thus of their possible use in the nutritional field, we believe it is useful to report some analytical data obtained in the examination of some tall oil samples imported from Germany, Sweden and the United States (see Table A). The values of the analytical constants reported here represent averages of values obtained in the analysis of numerous samples; in fact, we were able to determine that the same values are not always obtained even when using samples having the same content (in percent) of resin acids and fatty acids. The slight differences encountered depend on the type of wood used, the region of its origin and the production process.

Table A

Constants of some distilled tall oils and crude tall oil

Tall oil type and reference No.	Refracto-metric degree at 25°C	Refractive index at 25°C	Iodine No. Wyis	Acid No.	Saponi-fication No.	Resin acids %	Fatty acids %	Unsapo-nifiables %
1. Tall oil fatty acids	62	1,4690	132	195	197	1	97	2
2. Tall oil fatty acids	63	1,470	134	194	196	2	95,5	2,5
3. Tall oil fatty acids	69	1,4715	136	192	193	3,5	93,5	3
4. Tall oil fatty acids	74	1,4770	135	185	195	5	93	2
5. Tall oil fatty acids	83	1,482	128	185	190	10	88,5	1,5
6. Distilled tall oil	85	1,483	125	178	188	15	84	1
7. Distilled tall oil	89	1,485	135	188	190	21	77,5	1,5
8. Distilled tall oil	93	1,488	158	190	192	32	66,5	1,5
9. Crude tall oil	—	—	168	168	175	45	50	5

In addition, whereas commercially the designation "tall oil fatty acids" refers to distilled tall oils containing up to 40% resin acids, the same designation, in our understanding, includes distilled tall oils containing up to 10% resin acids. In fact, the Ministry of Finance, concerned with the spread of fraud in the field of edible oils and anticipating the use of tall oil derivatives also in the nutritional field, decreed as far back as 1956 that distilled tall oils containing an amount of resin acids equal or inferior to 10% should be classified, from a customs service standpoint, under the term of Tariff 145b: "Fatty acids" and therefore subject to the fiscal rule established by D. L. (legal decree?) of October 1956, No. 1194.

The resin acids present in the various tall oil samples were determined by chemical means. For this purpose, various methods are known, such as the methods of Wolff (8), A. Linder and V. Person (9), Herrlinger and Compeau (10), and Mc Nicol (11).

The best results were obtained by using the method of Herrlinger and Compeau for tall oil with a content of resin acids not greater than 15%, and the method of Mc Nicol for tall oil with a resin acid content greater than 15%. In fact, Wolff's method does not give good results for tall oil containing small amounts of resin acids, and the method of A. Linder and V. Person has the same inconvenience, although it certainly represents a progress over other methods since it introduces empirical corrective terms into its formula. Tall oil unsaponifiables were determined by the ASTM-D1065 method.

The percentage of fatty acids present in tall oil is calculated by the formula:

$$\% \text{ fatty acids} = 100-(R + I)$$

where R is the percentage of resin acids, and I is the percentage of insaponifiables.

Tall oil samples No. 1-8 (see Table A) are oily liquids of a straw-yellow color, the intensity of which increases slightly with increasing content of resin acids. The odor is that of a fatty substance for the first 5 samples; starting with sample No. 6, a characteristic resinous odor is perceived, which increases slightly in the other samples.

Sample No. 9 is a crude tall oil, having the appearance of an oily liquid of greater density than the other samples and frequently containing a crystalline deposit of resin acids with a strongly resinous odor. The average specific weight (density) of the various tall oil samples ranges from a minimum of 0.900 to a maximum of 0.985.

Examination of Table A shows that the refractometric degree and the refractive index of the various samples increase with increasing content of resin acids; this observation is quite useful for the purpose of a customs classification of tall oil, since the percentage content of resin acids can be determined with good approximation in a short time.

It is interesting now to examine the analytical data pertaining to the fatty acids of tall oil sample No. 1 in Table A. We can see that these constants are very close to those of fatty acids present in soybean oil. In fact, tall oil fatty acids still containing 1% resin acids have the following average composition: 50% aleic acid, 48% linoleic acid, and 2% saturated fatty acids.

If we now compare (Table B) the composition (in percent) of these tall oil fatty acids with that of soybean oil fatty acids, we note a great similarity in the composition of these acids.

Table B

	Resin acids %	Linoleic acid %	Linolenic acid %	Oleic acid %	Saturated acids %
Tall oil fatty acids	1	48	0,5-1	50	2
Soybean oil fatty acids (12)	absent	54	5	28	12,7

By subjecting such tall oil fatty acids to esterification with glycerol an oil is obtained which, after refining, could possibly be used for nutritional consumption as a seed oil. However, with the same esterified oil, eventually mixed with other animal or vegetable oils with low indices, it is possible to obtain a commercial oil such as "olive oil", in which its chemical-physical constants can always be included into the very wide constants presently accepted for the so-called "Rectified olive oils B". Even a positive Morawski reaction is obtained, that is a characteristic reaction for the classical "Rectified olive oil B", which should consist of "oil extracted with solvents from olive husks, made edible by means of chemical manipulations, etc."

However, there is even more to it. Fraud can be carried out with major scientific strictness by using as raw material also any kind of distilled tall oil. In fact, since modern methods for the separation of single (individual) fatty acids are available today (13), it is possible to isolate those fatty acids which are of major interest and to prepare from them an esterified product with the characteristics of rectified olive oil B, and it is probably impossible to trace back the true origin of this product by means presently available to us.

The incentive for such a new type of fraud is provided naturally by the low cost of the raw material. The cost of crude tall oil does not exceed 80 lire/kg, and the cost of fatty aicds ranges at 150 lire/kg.

Since the fiscal rules to which various tall oil derivatives are subject today are not sufficient to prevent their fraudulent use in the nutritional field, the only course remaining is to place controls on the fabrication of esterified oils and fats. The enactment of such a measure is a necessity which cannot be delayed any longer by this time, if we wish to protect effectively the health and the economy of consumers and the future of our olive-tree cultivation.

Today, esterified oils prepared from a wide variety of raw materials are sold under the designation "Rectified olive oil B". And since the law of 1936, which is the primary cause of so many ills, allows the sale of a mixture of rectified product B and pure olive oil as "Olive oil", without specifying the ratio of this mixture, we have a commercial product sold under the designation of "olive oil" which consists to a great extent, in the most optimistic estimate, of a mixture containing 90% esterified oil and 10% olive pulp.

The 1936 law, in establishing the classification of olive oils, has been truly inconsistent even during those days when esterified oils were not available. In fact, this law claims the designation "olive oil" both for olive oil obtained from pressing olives, provided that it has an acidity between 2.5 and 4, as well as for a mixture consisting of "Rectified olive oil B" and pure olive oil. And since, as was stated above, the ratio of this mixture is not specified, the same designation includes both virgin olive oil as well as oils obtained by chemical manipulations. This problem has been aggravated in recent years for different reasons.

An industrial chemical process not contemplated in the law of 1936 has been in fact legalized. Indeed, not only has the direct esterification with glycerol of low-acidity olive husk oils been officially admitted, but also the esterification of fatty acids present in olive oil.

In this respect, Prof. D'Ambrosio has justly made the following declaration at a recent meeting in Milano: "The law of 1936 reserves the qualification of rectified olive oil B to those oils obtained by processing, even with chemical means, of olive pulp oils. In other words, in spite of whatever amplification made in the interpretation of the present law, the limits are determined by an element of incontrovertible and unequivocal fact, which applies namely, always and only, to oils and that these oils remain at every and any moment of the industrial phase. Therefore, it is obvious that the entire industrial activity of indirect esterification, that is to say the activity affecting fatty acids, which certainly are not oils, is contrary to the existing decrees of law".

However, from a practical standpoint we can go even further because, since it is not possible to determine analytically whether an esterified oil is derived from olive oil fatty acids or from fatty acids of another origin, we end up by approving the legal sale, under the designation of olive oil, also of an esterified oil which has nothing in common with olive oil. Vitagliano (14), when stating that olive oil is the only edible oil subjected to re-esterification and that this process is completely ignored by the seed oil industry, is perhaps unaware of what is going on today in the field of edible oils and fats.

In fact, the esterification process is undergoing continuous development. Large amounts of seed oils are being produced by esterification; in 1957, several hundred thousand kilograms of such oils were produced with fatty acids especially imported from West Germany.

Cocoa butter for comestible use is prepared by esterification of coconut and palm oil fatty acids. "Pscudo fats" (substitute fats) are prepared by esterification of fatty acids with ethylene glycol rather than glycerol (15) And, as Doro (16) affirms, perhaps the time has already come for the appearance of a synthetic oil prepared with fatty acids obtained by oxidation of paraffin hydrocarbons derived from coal by the Fischer-Tropsch synthesis.

In conclusion, let us remember that the time has come to impose controls on the production and sale of oils and fats obtained by esterification and to review the official classification of olive oils established by R. D. L. (?) of 27 September 1936, No. 1986. This is a requirement of a moral, economic and sanitary order.

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Gli acidi grassi del tallol ed il loro impiego in campo alimentare

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Le frodi nel campo degli oli vegetali commestibili vanno sempre più dilagando e ormai l'industria chimica è in grado di preparare oli vegetali partendo dalle più disparate materie prime. Infatti di recente è stato accertato che gli acidi grassi provenienti dal tallol vengono utilizzati per la produzione di oli esterificati che possono essere immessi al consumo alimentare direttamente come tali o in miscele con altri oli di semi o con olio di oliva.

Il tallol (Svedese tall-pino) è un sottoprodotto della preparazione della cellulosa al solfato dal legno di pino e la sua produzione ha avuto durante e dopo la ultima guerra un forte sviluppo specialmente negli U.S.A. per soddisfare al sempre crescente bisogno di oli essiccativi e quindi di acidi grassi non saturi per la produzione di resine alchidiche, pitture, vernici, saponi, ecc. ecc.

Nella preparazione della cellulosa al solfato si forma un liquido nero, sciropposo, (« sapone del liquore nero ») da cui, per azione dell'acido solforico, lavaggi con acqua e decantazione, si ottiene il tallol greggio costituito da una miscela di acidi grassi, acidi resinici e sostanze insaponificabili. Le percentuali di tali componenti variano secondo il tipo di legno usato e la sua regione di origine. Ma l'industria riesce a ridurre al minimo tali variazioni, ricorrendo a opportune miscele e mette così il commercio dei tallol greggi in cui le percentuali degli acidi grassi e resinici variano in più o in meno del 2-3% e l'insaponificabile dell'1% al massimo.

Poichè i singoli componenti del tallol greggio hanno avuto un sempre maggiore campo di applicazione, l'industria chimica, sottoponendo il tallol greggio a distillazioni frazionate, è riuscita a produrre diversi tipi di tallol distillati (con diverse percentuali di acidi grassi e resinici) fino ad ottenere acidi grassi quasi privi di acidi resinici (1%) e acidi resinici quasi privi di acidi grassi (1%).

Il tallol greggio secondo Hezel (1) è costituito dal 30-50% di acidi resinici, 60-45% di acidi grassi, 1-6% di acidi grassi e resinici ossidati, 1-10% di insaponificabile.

Gli acidi grassi del tallol sono costituiti principalmente da acido oleico, linoleico e piccole quantità di acidi grassi saturi fra cui il palmitico e il lignocerinico. H. Niessen (2) fra gli acidi non saturi ha identificato con sicurezza anche il linolenico mentre Browning e Calkin (3) fra i non saturi ricordano il ricinoleico e il brassidico, e, fra i saturi, il palmitico, il laurico, il miristico e il lignocerinico.

Gli acidi resinici del tallol sono simili a quelli della colofonia e F. J. Ball e W. J. Vardell (4) con analisi spettrofotometrica hanno dimostrato che circa la metà degli acidi resinici è costituita da acido abietico e neoabietico. Sono inoltre presenti altri acidi resinici che differiscono dall'acido abietico nel numero o nella posizione dei doppi legami o nella struttura della catena: acido deidroabietico, tetraidroabietico, d-pimarico, isod-pimarico.

L'insaponificabile contenuto nel tallol è costituito principalmente da una miscela di idrocarburi, alcoli a lunga catena e steroli. Fra gli idrocarburi sono presenti l'abietene, i diterpeni, i polimeri terpenici e i polimeri degli acidi resinici decarbossilati; fra gli alcoli, l'alcole lignocerilico e fra gli steroli il beta-sitosterolo.

Naturalmente durante la distillazione del tallol le sostanze insaponificabili distillano a seconda del loro punto di ebollizione.

L'abietene passa con le frazioni di testa, gli steroli e gli altri alcoli a punto di ebollizione più alto rimangono nella pece di tallol. Pertanto i tallol distillati e gli acidi grassi di tallol sono privi di steroli e di alcoli ad alto punto di ebollizione. Jacini (5) in uno studio sull'utilizzazione del tallol e sull'isolamento di alcune sterine, afferma che non ci sono dubbi sulla identità delle fitosterine da tallol con la fitosterina ricavata da altri vegetali (es. soja) e in un altro studio (6) sulla pece di un tallol italiano, contrariamente a quanto affermato da Sandquist, non ha trovato alcole lignocerico ma alcole arachico normale.

La pece di tallol, ricca in steroli, è diventata quindi una materia prima importante per la produzione del beta-sitosterolo impiegato nella sintesi dei sei ormoni e di altri composti biologicamente attivi. Sempre Jacini (7) descrive un metodo pratico di estrazione delle sterine dal « sapone solfatico » del tallol e la successiva ossidazione e deidroandrosterone della miscela tecnica, ottenuta dalle sterine non particolarmente purificata o frazionata.

Da quanto sopra è evidente che l'importanza del tallol, viene accresciuta fortemente per il fatto che esso ci offre la possibilità di utilizzare le sterine di origine vegetale, al posto della colesterina, nella preparazione di alcuni ormoni, partendo da materie prime di basso costo.

Le applicazioni industriali del tallol greggio e dei suoi derivati sono quindi oggi numerose e sicuramente l'industria chimica riuscirà con il tempo ad ampliare il loro già vasto campo di impiego.

Che però l'industria utilizzasse tali prodotti anche in campo alimentare, fino a due o tre anni fa non era pensabile. Purtroppo oggi tale utilizzazione è divenuta una amara realtà e l'incentivo a tale illecito uso è il costo di tali prodotti, notevolmente inferiore a quello degli oli vegetali naturali.

Per una migliore conoscenza della composizione dei derivati del tallol e quindi della possibilità del loro impiego in campo alimentare, riteniamo utile riportare (Tabella A) i dati analitici ottenuti dall'esame di alcuni campioni di tallol importati dalla Germania, Svezia e Stati Uniti. I valori delle costanti analitiche riportate rappresentano la media dei valori ottenuti dall'analisi di numerosissimi campioni; infatti abbiamo potuto constatare che non sempre si ottengono i medesimi valori pur partendo da campioni aventi una stessa percentuale di acidi resinici e di acidi grassi. Le lievi differenze riscontrate dipendono dal tipo di legno impiegato, dalla regione di origine e dal processo di fabbricazione.

Inoltre, mentre commercialmente la denominazione di « acidi grassi del Tallol » si riferisce ai tallol distillati che contengono fino al 4% di acidi resinici, noi sotto la stessa denominazione comprendiamo i tallol distillati contenenti fino al 10% di acidi resinici. Infatti il Ministero delle Finanze, preoccupato del dilagare delle frodi nel campo degli oli alimentari e prevedendo una utilizzazione in campo alimentare anche dei derivati del tallol, stabilì fin dal 1956 che i tallol distillati contenenti una percentuale di acidi resinici uguale o inferiore a 10 venissero doganalmente classificati sotto la voce di Tariffa 145-b: « Acidi grassi » e pertanto soggetti alla disciplina fiscale stabilita con il D. L. 31 ottobre 1956, n. 1194.

Gli acidi resinici dei vari campioni di tallol sono stati determinati per via chimica. A tale scopo esistono vari metodi: di Wolff (8), di A. Linder e V. Person (9), di Herrlinger e Compeau (10), di Mc. Nicol (11).

I migliori risultati si sono ottenuti seguendo il metodo Herrlinger e Compeau per tallol con un contenuto in acidi resinici non superiore al 15%, ed il metodo Mc. Nicol per tallol con più del 15% di acidi resinici. Infatti il metodo Wolff non dà buoni risultati per tallol contenenti piccole percentuali di acidi resinici e lo stesso inconveniente presenta il metodo di A. Linder e V. Person sebbene esso costituisca certamente un progresso rispetto ad altri metodi perchè nella formula introduce dei termini correttivi empirici.

L'insaponificabile dei tallol è stato determinato con il metodo A S T M - D 1065.

La percentuale degli acidi grassi contenuti in un tallol è data da:

$$\% \text{ acidi grassi} = 100 - (R + I)$$

dove: R = percentuale degli acidi resinici
I = percentuale dell'insaponificabile

I campioni dal n. 1 al n. 8 hanno l'aspetto di liquidi oleosi, di colore giallo paglierino che si intensifica leggermente con l'aumentare degli acidi resinici.

TABELLA A

Costanti di alcuni tipi di tallol distillati e di un tallol greggio

Tipo di tallol e numero di riferimento	Grado rifrattometrico a 25°	Indice di rifrazione a 25°	N. I. Wyis	N. A.	N. S.	Acidi resinici %	Acidi grassi %	Insaponificabile %
1. Ac. grassi di tallol	62	1,4690	132	195	197	1	97	2
2. Ac. grassi di tallol	63	1,470	134	194	196	2	95,5	2,5
3. Ac. grassi di tallol	69	1,4715	136	192	193	3,5	93,5	3
4. Ac. grassi di tallol	74	1,4770	135	185	195	5	93	2
5. Ac. grassi di tallol	83	1,482	128	185	190	10	88,5	1,5
6. Tallol distillato	85	1,483	125	178	188	15	84	1
7. Tallol distillato	89	1,485	135	188	190	21	77,5	1,5
8. Tallol distillato	93	1,488	158	190	192	32	66,5	1,5
9. Tallol greggio	—	—	168	168	175	45	50	5

L'odore è quello di una sostanza grassa per i primi 5 campioni; col campione n. 6 si comincia a sentire il caratteristico odore resinoso, che va leggermente aumentando negli altri campioni.

Il n. 9 è un tallol greggio, che si presenta come un liquido oleoso più denso degli altri e che frequentemente ha un deposito di cristalli di acidi resinici, con odore fortemente resinoso. Il peso specifico dei vari tipi di tallol in media va da un minimo di 0,900 ad un massimo di 0,985.

Dall'esame della tabella si rileva che il grado rifrattometrico e l'indice di rifrazione dei vari campioni aumentano con l'aumentare degli acidi resinici e tale constatazione è molto utile agli effetti della classificazione doganale del tallol, perchè in breve tempo si può con una buona approssimazione stabilire il contenuto percentuale degli acidi resinici.

E' interessante ora soffermarci sui dati analitici riferentesi al campione di acidi grassi riportati al n. 1 della tabella A. Si vedrà che tali costanti sono molto vicine a quelle degli acidi grassi dell'olio di soja. Infatti gli acidi grassi del tallol contenenti ancora l'1% di acidi resinici hanno in media la seguente composizione: acido oleico 50%; acido linoleico 48%; acidi grassi saturi 2%.

Se ora confrontiamo (Tabella B) la composizione centesimale di tali acidi grassi del tallol con quella degli acidi grassi dell'olio di soja, notiamo che vi è una grande analogia fra loro.

Sottoponendo ad esterificazione con glicerina tali acidi grassi del tallol si otterrà un olio che dopo raffinazione potrebbe essere immesso al consumo alimentare come olio di semi. Ma con lo stesso olio esterificato, miscelato opportunamente con altri oli di origine animale o vegetale a bassi indici, si può ottenere un olio commerciabile come « olio di oliva » in quanto le sue costanti chimico-fisiche potranno sempre rientrare fra quelle, molto ampie, oggi accettate per i cosiddetti « Oli di oliva rettificati B ». Anzi darà positiva anche la Morawski, reazione questa caratteristica del classico « Olio di oliva rettificato B » che dovrebbe essere costituito « da olio estratto con solventi dalla sansa di oliva, *reso commestibile* mediante manipolazioni chimiche ecc., ecc. ».

Ma c'è di più. La frode può essere attuata con maggior rigore scientifico usando come materia prima anche un qualsiasi tipo di tallol distillato. Infatti, poichè esistono oggi moderni metodi di separazione dei singoli acidi grassi (13), si possono isolare gli acidi grassi che più interessano e preparare con essi un esterificato con le caratteristiche dell'olio di oliva rettificato B e non è possibile forse con i mezzi attuali risalire alla sua vera origine.

L'incentivo a tale nuova forma di frode è naturalmente dato dal basso costo delle materie prime. I tallol greggi hanno un costo che non supera le 80 lire al kg. e quello degli acidi grassi si aggira sulle 150 lire.

E poichè la disciplina fiscale cui sono oggi sottoposti i vari derivati del tallol non è sufficiente ad impedire il loro impiego fraudolento in campo alimentare, non rimane che porre sotto controllo la fabbricazione degli oli e dei grassi esterificati.

L'emanazione di tale provvedimento è ormai una necessità improrogabile, se si vuole effettivamente proteggere la salute e l'economia del consumatore e l'avvenire della nostra olivicoltura.

Oggi gli oli esterificati, preparati con le più diverse materie prime, vengono venduti con la denominazione di « Olio di oliva rettificato B ». E poichè la legge del 1936, causa prima di tanti mali, permette di vendere come « Olio di oliva » una miscela di rettificato B con olio fino di oliva senza stabilire i rapporti della miscela, abbiamo in commercio sotto la denominazione di « Olio di oliva » un prodotto che in notevoli quantità è costituito, nella più rosea delle ipotesi, da una miscela del 90% di esterificato e 10% di polpa di oliva.

La legge 1936, nello stabilire la classifica degli oli di oliva è stata veramente incongruente anche per quei tempi in cui non esistevano gli esterificati. Infatti tale legge riserva la denominazione di « Olio di oliva » sia all'olio di oliva ottenuto per pressione dalle olive, purchè abbia una acidità compresa fra 2,5 e 4, sia ad una miscela costituita da « Olio di oliva rettificato B » con olio fino di oliva. E poichè come si è già detto, non stabilisce i rapporti di detta miscela, la stessa denominazione vale sia per un *olio di oliva vergine* che per un olio ottenuto *con manipolazioni chimiche*.

Il problema si è aggravato in questi ultimi anni per diverse ragioni.

Un processo chimico industriale non contemplato nella legge del 1936 è stato di fatto legalizzato. Infatti non solo è stata ammessa ufficialmente la esterificazione diretta con glicerina di oli di sansa a bassa acidità ma anche la esterificazione di acidi grassi di olio di oliva.

E a questo proposito il Prof. D'Ambrosio in un recente convegno tenuto a Milano ha giustamente dichiarato: « La legge del 1936 riserva la qualifica di olio di oliva rettificato B a quegli oli ottenuti dalla lavorazione, anche con mezzi chimici, degli oli di sansa. In altre parole, malgrado qualunque dilatazione

TABELLA B

	Ac. resinici %	Ac. linoleico %	Ac. linolenico %	Ac. oleico %	Ac. saturi %
Acidi grassi del tallol	1	48	0,5-1	50	2
Acidi grassi dell'olio di soja (12)	assente	54	5	28	12,7

nell'interpretazione della legge attuale, il limite è stabilito da un elemento di fatto incontrovertibile e inequivocabile, che si agisca cioè, sempre e soltanto, sugli oli e che tali restino in ogni e qualsiasi momento della fase industriale. E' ovvio quindi che tutta l'attività industriale di esterificazione indiretta, vale a dire quella operante sugli acidi grassi, che certamente non sono oli, è contraria alle vigenti disposizioni di legge ».

Praticamente però si va ancora oltre perchè, dato che non è possibile analiticamente stabilire se un olio esterificato proviene da acidi grassi di olio di oliva o da acidi grassi di altra origine, si finisce con il consentire la vendita legale con la denominazione di olio di oliva anche di un olio esterificato che non ha nulla a che vedere con l'olio di oliva. Vitagliano (14) quando afferma che l'olio di oliva è l'unico olio alimentare ad essere sottoposto alla riesterificazione e che l'industria degli oli di semi la ignora completamente, non è forse al corrente di quanto oggi avviene nel campo degli oli e grassi alimentari.

Infatti il processo di esterificazione è in continuo sviluppo. Forti quantitativi di oli di semi vengono preparati con l'esterificazione. Nel 1957 ne sono stati prodotti diecine di migliaia di quintali con acidi grassi importati specialmente dalla Germania Occidentale.

Si prepara burro di cacao, per uso alimentare, esterificando acidi grassi di cocco e di palma.

Si preparano « pseudo-grassi » per esterificazione di acidi grassi con glicole etilenico anzichè con glicerina (15). E, come afferma Doro (16), è forse già arrivato l'olio sintetico preparato con acidi grassi ottenuti dalla ossidazione di idrocarburi paraffinati provenienti dal carbone mediante la sintesi Fischer-Tropsch.

Per concludere riteniamo che sia giunto il momento di porre sotto controllo la produzione e la vendita degli oli e grassi ottenuti per esterificazione e di rivedere la classifica ufficiale degli oli di oliva stabilita con il R. D. L. 27 settembre 1936, n. 1986.

E' questa una esigenza di ordine morale, economico e sanitario.

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144

STUDIES ON THE USE OF TALL OIL FATTY ACIDS IN THE DIET OF RATS

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SUOMALAINEN TIEDEAKATEMIA

Contents

132

Abstract

The nutritional properties of tall oil fatty acids have been investigated using rats as experimental animals. The effects of tall oil fatty acid distillate and its ethyl and glyceryl esters on the growth rate, food consumption, reproduction and longevity of the rats, on the absorbability and on the fatty acid compositions of adipose tissue, plasma, liver and fecal lipid fractions were investigated.

Mainly weanling male rats were used in the growth and absorbability experiments and both male and female rats in long-term and reproduction experiments. The organs from which lipids were extracted for the determination of fatty acid compositions were from full-grown rats. The semisynthetic diets that were given to the animals contained fat at levels of 15, 30 or 60% of total calories.

The results indicated that tall oil fatty acids contain some toxic factor, the effects of which were a retardation of growth, disorders of the skin and fur, and even death when the tall oil fatty acid content of the diet was 60 cal %.

When more effectively refined tall oil glycerides were fed, the growth-retarding effect of the toxic factor was clearly weaker. Hydrogenation of tall oil fatty acid glycerides apparently improved the nutritional properties of the product.

Histopathological investigations of animals fed hydrogenated tall oil fatty acid glycerides revealed a couple of cases of thyreoiditis and a few cases of degenerated liver parenchyma and swollen tubular cells, which were, however, relatively minor.

Determination of the absorbability of the tall oil fatty acid preparations tested indicated that they were absorbed to the extent of 93–96%.

The growth-retarding factor is evidently produced from the highly unsaturated fatty acids in the tall oil by the elevated temperatures used in the pulping and tall oil distillation processes.

Gas-chromatographic determinations of the fatty acid compositions of the adipose tissue, plasma, liver and fecal lipids from rats given tall oil fatty acid distillate showed that *cis*-5,9,12-octadecatrienoic acid, which is a typical tall oil fatty acid, accumulated in the fecal lipids. Only minor amounts of this acid were detected in the organ lipids.

I. INTRODUCTION

Tall oil, which is a by-product of the sulphate pulp manufacturing process, is a mixture of resin and fatty acids. As the cellulose industry has expanded, the amount of crude tall oil produced has risen appreciably in the last few years. A large part is exported in crude and refined grades. The crude tall oil can be separated into resin acid and fatty acid fractions by distillation. The resin acids are used almost entirely by the domestic paper industry whereas the fatty acid fraction is used primar[illegible] the paint and detergent industries.

The tall oil fatty acid distillate resembles several seed oils in fatty acid composition. The main components are oleic and linoleic acids, which alone comprise about four fifths of the total acids. The unique feature of tall oil is the presence of *cis*-5,*cis*-9,*cis*-12-octadecatrienoic and *cis*-5,*cis*-11,*cis*-14-eicosatrienoic acids since the locations of the double bonds in these acids differ from the locations of the double bonds in the octadecatrienoic and eicosatrienoic acids of natural fats. The presence of physiologically important C_{18} polyethenoids in tall oil has led to the assumption that tall oil fatty acid distillate may prove to be an economically advantageous domestic source of these acids for the manufacture of fodder and food fats.

The effect of tall oil fatty acids incorporated in the fodder of dairy stock and poultry has been extensively investigated by M. Antila and V. Antila and coworkers.

The present study was undertaken to determine the effects of tall oil fatty acids on albino rats. Growth, food consumption, reproduction and longevity experiments were carried out with the rats which were given different amounts of tall oil fatty acids as such or as their ethyl and glyceryl esters. In addition, the fatty acid compositions of some organ lipids were determined. Dietary fats are metabolized or stored in different tissues according to their roles in lipid metabolism. An attempt was made to follow the fate of the *cis*-5,9,12-octadecatrienoic acid in the lipids of rat adipose tissue, blood plasma, liver and feces.

II. TALL OIL

A. The isolation of crude tall oil

The alkaline sulphate pulping process, which is known as the Kraft process, in addition to its primary purpose of dissolving the lignin which binds the cellulose fibers together, also saponifies the fatty acids and resins of wood. Since pine wood, which is rich in resins, is the most important raw material, fatty acid and resin soaps are produced in large quantities. The soap is contained in the spent cooking liquor, which is known as black liquor. After pulping, the cellulose is washed to separate the black liquor from the pulp as effectively as possible. The black liquor is usually concentrated in multiple-effect evaporators to a point where it can be burned in special boilers for the recovery of inorganic salts and the production of steam. After the evaporation stage, when the black liquor has been concentrated to a solids content of 25–30%, it is passed to soap skimmers where the major portion of the soap is salted out and rises to the surface from which it is skimmed off. The skimmings contain 60% soap, which is the starting material for the production of crude tall oil.[1,2]

When the sulphate pulping process was developed in 1884, there was hardly any interest in the recovery of the fatty matter in the wood. The black tarry soap was washed from the pulp and discarded. Later it was found that this material could be burned for the recovery of inorganic salts. In 1895 Lönneberg separated tall oil from the black liquor by adding acid or salt.[3] At the beginning of this century tall oil was recognized as a valuable by-product of the pulping industry. The development of tall oil production was not very rapid before the end of the Second World War, but the manufacture and utilization of tall oil have subsequently increased at a phenomenal rate.

The original method of isolating crude tall oil, which consisted in treating the black liquor skimmings with dilute sulphuric acid, was a batch process. The reaction was carried out in a large wooden tank at temperatures of 93—99°C; this batch process is still used with minor improvements. Steam is used for heating and agitating the mass. The acidulated mixture is then allowed to settle by gravity and the crude tall oil is decanted off, washed with sodium sulphate solution or water to remove sulphuric acid residues and then heated to remove the water. The residue contains, in addition to spent sulphuric acid, lignin derivatives and inorganic salts. The wash solution is drawn off and combined with the alkali recovery liquors of the pulp mill. The batch process is preferred by small factories, since maintenance and operation costs are low. The tall oil recovered in the batch process amounts to about 88—90% of the available oil.[2,4]

A semicontinuous process for the recovery of tall oil was developed in the early 1950's. In this process the acidification was done batchwise, but the separation of the crude tall oil from the spent acids and lignin derivatives was carried out continuously in a nozzle-type centrifuge. The chief advantage of this process is the elimination of the gravity settler with a correspondingly higher yield, 96—97%, and an improved quality of crude tall oil.[2]

Sullivan[5] developed a continuous process in which a proportioned amount of dilute sulphuric acid is added continuously to the settled black liquor skimmings. This acidulated mixture is screened to remove fibers and gases such as hydrogen sulphide, mercaptans and sulphur dioxide produced in the reaction. After this screening, the mixture is separated continuously in a nozzle-type centrifuge. Several improvements have been made in the basic process in the last few years. These include more accurate proportioning of the reagents and controlled mixing and degasification.[2,6] The introduction of a new type of centrifuge known as the self-cleaning centrifuge has made the separation of crude tall oil even more economical and effective. This is a big advantage over the old nozzle-type centrifuge, where fibers caused much plugging.[3]

The amount of crude tall oil produced in Finland has steadily increased along with the growing pulp industry and was about 76 000 tons in 1967. This cannot be exceeded very much in coming years, because the availability of wood for pulping is limited. Over 23 000 tons of tall oil was exported, the domestic use of unrefined tall oil was 5 700 tons, and the rest was refined by distillation.[7] It may be mentioned that the world production of crude tall oil is estimated to be about 750 000 tons of which the United States alone produce about 500 000 tons. These estimates do not include the production in Eastern bloc countries, because no reliable statistics are available. The average yield of crude tall oil per ton of pulp is 30 kg in Finland, but the highest yields have been over 60 kg of tall oil per ton of pulp.[8,9] In Sweden the maximum yield is 70 kg per ton and in the United States the maximum yield is even higher, 100—120 kg per ton of pulp.[3]

B. Composition of crude tall oil

The compositions of crude Finnish tall oils are according to Elovaara[8]:

Fatty acids	40—70%
Resin acids	30—45%
Unsaponifiable matter (neutral substances)	5—20%

The following range of composition of American crude tall oil from a continuous acidulation system is given by Thrush[2]:

Fatty acids (mainly C_{18} acids)	30—35%
Saturated fatty acids	5— 7%
Resin acids	35—60%
Unsaponifiables	5—10%

As shown by these figures, there are great differences in the compositions of crude tall oils. Several factors affect the composition; such are the geographic site of growth, the season when timber is felled and the time and method of storage of wood. The yields of tall oil are higher in northern Finland than in the southern part of the country, and also the resin acid content of the tall oil is higher in the north.[8] There is also a difference in fatty acid composition in that the fatty acids in the tall oil from northern Finland include more unsaturated C_{18} acids, which makes the Finnish tall oil more valuable than, e.g., North American tall oil. Sarkanen and Kahila[10] determined the compositions of several crude tall oil samples from northern and southern Finland. The ranges of the fatty acid fractions were found to be as follows:

	Northern Finland	Southern Finland
Saturated acids	6.3– 6.8%	8.3–18.3%
Oleic acid	28.0–37.0%	32.0–43.0%
Linoleic acid	55.0–64.0%	38.0–57.0%
Linolenic acid	0.9– 2.0%	0.5– 1.0%

Several investigations have dealt with the effect of storage of wood on the yield and composition of crude tall oil. Kahila[11] investigated the effect of a storage period of five years on land and in water. The yield of tall oil gradually diminished to about 50% on storage on land and to about 70% on storage in water. Cowart *et. al.*[12] found a storage time of 12 weeks to reduce the yield of tall oil from pine roundwood approximately 11%; during the same time the reduction in the yield of tall oil from slab-wood pine chips was 64%. The loss was predominantly due to loss of fatty acids. Nordin and Selleby[13] investigated the effect of storage time on chips made from either green wood or stored roundwood and found that the yield of crude tall oil from green wood chips was considerably lower than the yield of tall oil from roundwood chips stored for the same time. If the storage took place during the cold season, the reduction in tall oil yield from the roundwood was considerably smaller, but indoor storage (20°C) led to almost the same rate of tall oil loss as chip pile storage.

The main reason for the decrease in yield during storage of pulpwood is oxidation or some other modification of the wood extractives. Mainly the fatty and resin acids are modified in such a way that they become soluble in the black liquor and are not separated with the sulphate soap.[14]

The composition of the fatty acid fraction of tall oil is discussed in detail later in this chapter. The other components of crude tall oil are the resin acids and unsaponifiable matter. Pine wood extract contains large amounts of levopimaric, abietic and neoabietic acids, smaller amounts of dehydro-, dihydro-, dextro- and isodextropimaric acids, and only traces of tetrahydroabietic acid.[15] The pulping process and the decomposition of the soap skimmings with sulphuric acid changes the composition of resin acids greatly. Levopimaric acid disappears almost completely, the amount of abietic acid is doubled and the amount of dihydro- and tetra-hydroabietic acids increases even more, but the amount of the other acids remains unchanged. Resin acids of the abietic type contain a conjugated double bond system, which is missing in the pimaric type, and this structural difference is probably the reason for the difference in stability of the two types.

As mentioned, the total amount of unsaponifiable neutral substances in crude tall oil is 5—20%. The main components are hydrocarbons and sterols.[16] Lignoceryl and aracinal alcohol, α- and β-sitosterols, stigmasterols and aliphatic and alicyclic hydrocarbons have been isolated in pure form from the unsaponifiable matter.[3]

C. Distillation of tall oil fatty acids

Crude tall oil can be used as such for certain purposes, but generally it is distilled or refined in some other way in order to obtain fatty and resin acids as pure as possible.

For the separation of fatty acids and resin acids, methods based on the use of solvents, saponification,[17] partial esterification,[3] countercurrent distribution (Solexol process)[18] and crystallization of individual fatty acids[19,20,21] have been applied. Most of these methods have only theoretical significance. The most important method is without doubt the distillation of crude tall oil. So far as is known, the first distillation plant was built in Finland in 1912 by A. Hellström.[22] The main product of this establishment was distilled tall oil which consisted of both fatty and resin acids and which was used for the production of potassium tall oil soap.

Simple distillation can be used, but the separation of different fractions is incomplete,[23] and therefore fractional distillation has gradually replaced it in the tall oil industry all over the world. In Finland only fractional distillation is used.[8]

The distillation is carried out as quickly as possible and at as low a temperature as possible to prevent thermal decomposition. Particularly fatty acids decompose easily when subjected to high temperatures. The first step is usually the formation of anhydrides with loss of water. When the temperature is increased further, ketones and hydrocarbons are formed with loss of carbon dioxide. Unsaturated fatty acids form partly nonvolatile polymers and all these reactions together impair the quality of the tall oil and reduce the yield. Injected steam and reduced pressure are used to prevent thermal decomposition during distillation and also to suppress anhydride formation. The temperature is kept at approximately 250°C.[1] The main components of the fractional distillation plant of Oulu Oy are the following.[8] Four columns made of high quality acid-proof stainless steel are used for distillation. The columns are heated from the bottom with electric resistances and the vacuum is produced by a steam jet ejector. The pressure at the heads of the columns is only about 1 mmHg. The crude tall oil is pumped into the first column and the undistilled tall oil pitch, which contains the colored impurities of crude tall oil, is removed from the bottom of this column. The distillate from the top of the column contains the main part of the fatty and resin acids of the crude tall oil and some of the neutral substances. The distillate is fed to the next column, where the resin acids, which have higher boiling points than the fatty acids, remain on the bottom and are removed. The fatty acids and a small amount of resin acids and neutral substances rise to the top of the column and pass to the third column. The distillate from the third column is relatively small in volume and this tall light oil consists mainly of neutral substances and palmitic acid. The main part is passed to the fourth column, the so-called fatty acid column. From the top of this column some tall light oil distillate is fed back to the third column to maintain the purity of the main fraction as high as possible. In order to reduce the resin acid content of this fatty acid fraction to a minimum, the more resinous distillate is removed from the bottom of this column. Previously the resin acids were allowed to separate by crystallization and the yield was relatively high, about 97% of the resin acids, but nowadays the economical resin acid distillate is preferred to the more expensive crystalline resin in products.

The composition of the fatty acid distillate of Oulu Oy is as follows:

Fatty acids	more	than	95%
Resin acids	less	than	2%
Neutral substances	less	than	3%

The resin acid fraction contains 91% resin acids, 3% fatty acids and 6% neutral substances.

Barnes *et al.*[1] have given the following figures for an American tall oil fatty acid distillate:

Fatty acids	99.2%
Resin acids	0.5%
Unsaponifiable matter	0.6%

D. Composition of the tall oil fatty acid distillate

Structural and quantitative changes occur during the processing of the tall oil fatty acid distillate, so that its composition may vary considerably. According to different investigations, the proportion of saturated fatty acids, palmitic, stearic and myristic acids, varies between 5 and 11%, although even higher proportions have been observed. Palmitic acid dominates the saturated acids, During the distillation the saturated acids mainly distill with the tall light oil.

The main unsaturated components of tall oil fatty acids are unsaturated

C_{18} acids. The amount of oleic acid varies from 25 to 40% and that of linoleic acid from 35 to 50%. Linolenic acid had not been detected earlier,[24] but modern methods have revealed that there is 0.5–2% of linolenic acid.[16]

The fatty acid composition of Finnish tall oil fatty acid distillate has been extensively investigated during the last few years. The main reasons are the growing importance of the tall oil distillate in the chemical industry and the fact that modern analytical methods give more accurate results. Aho *et al.*[25] determined the composition of a tall oil distillate from the Yhtyneet Paperitehtaat Oy Valke. Their analytical results are presented in Table 1. A particularly interesting feature is the presence of almost 10% of *cis*-5,9,12-octadecatrienoic acid among the fatty acids. The chemical structure of this acid was determined by oxidative degradation with ozone, when fragments consisting of C_3, C_4 and C_5 dicarboxylic acids and a C_6 monocarboxylic acid were detected. The acid contains the exceptional grouping

Table 1.

The fatty acid composition of Finnish tall oil fatty acid distillate (Yhtyneet Paperitehtaat Oy Valke).[25]

Myristic acid	< 0.1%
Palmitic acid	1.4
Stearic acid	0.3
Palmitoleic acid	< 0.1
Oleic acid	40.9
Linoleic acid	38.4
Linolenic acid	< 0.1
Saturated C_{17} (branched)	1.2
Saturated C_{17}	0.1
Monounsaturated C_{17} (branched)	< 0.1
Monounsaturated C_{17}	< 0.1
Monounsaturated C_{19} (branched)	0.3
Monounsaturated C_{19}	< 0.1
Diunsaturated C_{17}	< 0.1
Diunsaturated C_{18}	1.5
Diunsaturated C_{19} (?)	1.1
Diunsaturated C_{20} (?)	0.7
Triunsaturated C_{18} (*cis*-5,9,12)	9.9
Triunsaturated C_{19}	0.6
Triunsaturated C_{19}	1.3
Triunsaturated C_{19}	1.1

$= CH-CH_2-CH_2-CH =$. Lehtinen *et al.*[26] separated the same acid from the fatty acid distillate from Oulu Oy. In 1963 a third group of Finnish investigators[27] confirmed the structure of this fatty acid. Tall oil fatty acid distillate contains also other fatty acids of exceptional structure. Lehtinen *et al.* isolated *cis*-5,9-octadecadienoic acid,[28] *cis*-5,11,14-eicosatrienoic acid,[29] *cis*-11-eicosenoic acid and a mixture of conjugated *trans-9,trans*-11-octadecadienoic and *trans-10,trans*-12-octadecadienoic acids.[30] The last two compounds are probably secondary and are formed during the sulphate pulping and tall oil distillation processes.

Assarson and Åkerlund[31] investigated the occurrence of *cis*-5,9,12-octadecatrienoic acid in different coniferous species and found that the extractives of spruce (*Picea excelsa*) contained about 29% and the extractives of pine (*Pinus silvestris*) 17%, whereas no *cis*-5,9,12-octadecatrienoic acid was found in juniper (*Juniperus communis*).

III. USE OF TALL OIL FATTY ACIDS IN FODDER AND FOOD

A. Earlier investigations

Because of their favorable price, tall oil fatty acids have been used in products where they excellently replace the more expensive linseed and soybean oils. To date there have been only few investigations into the possible use of tall oil fatty acids in the production of edible fats and animal fodders, but the problem has attracted great interest.

Sheely and Potts[32] considered the best quality fatty acids of tall oil to be so bland and light in color that if they would be esterified with glycerol, they might well make a good salad oil.

According to Paolini[33] tall oil fatty acid glycerides have been used to adulterate natural oils. These glycerides have been mixed with olive and other seed oils. Paolini mentions also that individual tall oil fatty acids have been separated by modern methods and esterified to oils, which have been sold as olive oil of B class. The reason for this is the low price of the tall oil fatty acids.

M. and V. Antila with coworkers have during several years investigated the suitability of the fatty acid distillate of tall oil and its derivatives, primarily the ethyl esters, as ingredients of different fodder mixtures for dairy stock and poultry. The reasons for investigating the problem have been partly economical and partly technical. In order to increase milk and egg production, foreign soybean and linseed oil cakes have been imported to Finland. At the same time the domestic wood-processing industry produces as a by-product tall oil fatty acid distillate of high quality which resembles the oils of many plants in fatty acid composition. On the other hand, as ethyl alcohol, a by-product of the manufacture of sulphite cellulose, is relatively cheap and the fatty acids of tall oil are relatively easily esterified with ethyl alcohol, the investigations were concentrated on a study of the properties of the ethyl esters of the distilled fatty acids.[34]

Another fact associated with this problem is the change in consistency of dairy butter during the indoor feeding period, when butter contains relatively low levels of unsaturated fatty acids.[35] It is known that by incorporating oils in fodder it is possible to keep the fat of the milk as soft during the indoor feeding period as during the pasture feeding period.

Antila *et al.*[35] used 3% of ethyl esters of tall oil fatty acids in fodder in their experiments with milch cows. If the amount was higher, it lowered slightly the milk production and the amount of milk fat. When ethyl esters of tall oil fatty acids were mixed with dried grass meal, the carotenoids of the grass meal probably acted as effective antioxidants and retarded the oxidation of the esters. The inclusion of ethyl esters of tall oil fatty acids in the fodder had no effect on the protein content of the milk, but led to a significant increase (10 units) in the iodine value of the milk fat. Determination of the fatty acid composition of the milk fat revealed that the amount of palmitic acid had decreased and that of oleic acid had increased. The observed increase in iodine value is probably due to this fact.[35, 36]

A separate experiment[37] was carried out in which tall oil fatty acid distillate was fed to cows at a level of 10% of the concentrates. The results revealed that this proportion raised the iodine value of the milk fat by 12 units. The fatty acid composition of the milk fat was changed so that saturated C_4 to C_{16} acids occurred in definitely lower proportions than before the experiment, whereas the proportion of C_{18} acids increased markedly.

The effect of ethyl esters of tall oil fatty acids in poultry feed mixtures on egg production and hatching was investigated by Antila *et al.*[38] During three experimental periods each lasting several months, hens were given dry feed mixtures containing 5 and 10% ethyl esters of tall oil fatty acids. The egg production decreased 15—16% in both tall oil fatty acid ester groups, while the decrease was only 5% in the control group given the dry feed mixture as such. The mixtures containing ethyl esters of tall oil fatty acids did not affect the fertilization and hatching of the eggs, but the baking characteristics were impaired. There was a change in the fatty acid composition of the egg yolk; the amount of oleic acid had increased considerably but the amount of palmitic, stearic and linoleic acids had correspondingly decreased. There were no essential changes in fat content or iodine value.

Costigliola and Teasdale have been granted two patents (Canadian patents Nos. 724 602 and 733 460)[39,40] for edible products containing tall oil fatty acid esters. The first patent describes the preparation of a salad oil with a high smoke point and long cold test properties. The method used to esterify the tall oil fatty acids to produce a triglyceride oil and to refine the product is almost the same as that described by Antila *et al.*[41,42] According to the patent specification, the resulting salad oil is odorless and has a good flavor stability, a relatively low free fatty acid content, a low residual content of resin acids and properties superior to salad oil made from, e.g., corn oil or soybean oil. Patent No. 733 460 describes the preparation of edible fats from tall oil fatty acids. The methods of esterification and hydrogenation are similar in principle to those presented by Antila *et al.*[41,42] The tall oil fatty acid triglycerides are blended or reacted with animal fats such as beef tallow and lard and vegetable oils such as palm oil, soybean oil and cottonseed oil to produce blended shortenings or margarine fats. As the tall oil fatty acid triglycerides contain over 70% triunsaturated triglycerides, the blended products can be modified to possess a higher content of unsaturated esters, a different melting range, a different solids content, etc.

B. Present Investigations

1. Materials and methods

a. Tall oil fatty acid distillate and its ethyl and glyceryl ester derivatives

The tall oil fatty acid distillate used in the experimental diets as such or after conversion into ethyl and glyceryl esters was obtained from Oulu Oy, Oulu. The fatty acid distillate »102» is a relatively high-grade product, but the contents of resin acids (1.8—2.2%) and unsaponifiable matter (2.8—3.2%) are rather high for a product to be used for nutritional purposes. Data relating to its chemical properties and fatty acid composition are summarized in Table 2.

With the exception of the pine seed oil samples and one lot of tall oil fatty acid glyceride margarine, which were prepared in the laboratory of the Department of Dairy Science, University of Helsinki, all ethyl and glyceryl esters and the fractions prepared from them which are described later and which were used in the feeding experiments were obtained from the Central Laboratory of the Raisio Factories, Raisio.

The pine seed oil was extracted from crushed pine seeds with hexane. The oil was recovered by distilling the hexane on a water bath.[43]

The ethyl esters of tall oil fatty acids were prepared by a continuous process.[41] The fatty acid fraction was esterified with ethyl alcohol using sulphuric acid (1—2%) as catalyst. The reaction temperature was 70—80°C. The crude esters were washed with water and dried under reduced pressure.

The glyceryl esters were prepared by esterifying the fatty acid distillate with glycerol using 0.2% stannous chloride as catalyst.[41] The water formed during the

Table 2.

Analytical data for the tall oil fatty acid distillate and soybean oil fatty acids.

	Tall oil fatty acids	Soybean oil fatty acids
Chemical properties:		
Acid value, mg KOH/g	190.2	180.7
Saponification value, mg KOH/g	194	190
Iodine value (Wijs)	150	138
Peroxide number	2.76	0.20
Fatty acid composition:		
C 12:0	—	0.1
C 14:0	0.5	0.3
C 16:0	1.0	10.7
C 16:1	—	0.6
C 18:0	1.0	2.8
C 18:1	38.8	20.5
C 18:2	42.8	53.6
C 18:3[1]	13.5	—
C 18:3	—	9.0
C 19:0	—	1.7
C 20:0	1.4	0.7

[1] *cis*-5,9,12-Octadecatrienoic acid.

reaction was removed by vacuum distillation, during which the temperature was raised to 200°C. The glyceryl esters were refined by the usual methods employed in the manufacture of edible oils. The analytical data given by Antila *et al.*[42] correspond to those of the starting material when the content of ethyl alcohol or glycerol in the esters is taken into account. The resin acid content of the ethyl esters was much lower than that of the original fatty acid distillate and only traces of resin acids were detected in the glyceryl esters.

A resin acid concentrate was separated from the glyceryl esters of tall oil fatty acids by the method of Linder and Persson[44] and the unsaponifiable matter was separated by the ASTM method.[45]

Hardened tall oil fatty acid glycerides were obtained by hydrogenation under reduced pressure. Nickel formate was used as catalyst. The melting point of the fat obtained was 36—38°C and the iodine number (Wijs) about 70.[41,42]

The hydrogenated tall oil fatty acid glycerides were interesterified with 20—25% refined soybean oil. Margarine was prepared from this fat mixture on a pilot plant scale. This tall oil fatty acid glyceride margarine was comparable with commercial margarine as to taste, flavor and consistency, but was relatively easily oxidized when stored.[42] One lot of tall oil fatty acid glyceride margarine was prepared in the Department of Dairy Science from hydrogenated tall oil fatty acid glycerides which had been prepared under normal atmospheric pressure at a temperature of about 100°C. The iodine value of the hydrogenated glyceride preparation was 80 (Wijs) and the melting point 50°C.

Molecular distillation of the glycerides of tall oil fatty acids was used in an attempt to isolate the unsaponifiable compounds.[46] A *cis*-5,9,12-octadecatrienoic acid concentrate was obtained by urea crystallization and further purification by countercurrent extraction. The fatty acid concentrate obtained, which contained about 75–80% of the mentioned fatty acid, was esterified with ethyl alcohol.[28]

The soybean oil used as a control fat in the feeding experiments was a commercial product of Raisio Oy, Raisio. The ethyl esters of soybean oil fatty acids were prepared by alcoholysis from soybean oil and hydrogenated soybean oil was produced by the same method as the hydrogenated glyceryl esters of tall oil fatty acids. The free fatty acids of the soybean oil were isolated according to Uksila.[47] The analytical data and fatty acid composition are summarized in Table 2.

The butter and margarine were commercial products.

b. *Experimental animals and their treatment*

Weanling male and female rats of the Sprague-Dawley strain, weighing 40–60 grams, were used in the growth experiments. The animals were provided by the Ylä-Mankkaa farm in Espoo, except those used in two of the long-term growth and reproduction experiments, which were bred in this laboratory. The animals were divided into groups having a similar average weight. In most growth experiments each group consisted of ten rats.

The rats were caged individually in suspended galvanized or steel wire bottom cages. The groups were distributed horizontally in the racks.

The animals were given food and tap water *ad libitum*. The food was distributed into glass dishes in the mornings. When the food consumption was recorded, 15–20 grams was weighed and the dish was covered with a galvanized lid with a small hole (diam. 2.5–3 cm) in the middle to reduce spillage. The following day, the leftovers and the waste were weighed and the food consumption calculated. The growth of the rats was recorded regularly by weighing them every day or every other day. The growth curves were obtained by plotting the cumulative weight gains against time.

The animals used in the reproduction experiments were between 100 and 200 days old. In the first reproduction experiment a pair of females was bred with one male, in the second experiment three females were bred with one male or four females with two males and in the third experiment the ratio was five females to two males. Farris[48] considers the optimum ratio to be two or three females to one male, but according to Hagemann[49] even 10–12 females can be bred with one or two males. After one week of breeding, every male was transferred to the next cage of the same experimental group. This was done in order to get the best breeding result in case some of the males were sterile. The males were removed after 14–16 days and the females were placed in separate cages before the 21st day following the original mating. The cages were either plastic cages whose floors were covered with wood shavings or wire cages with sheet aluminum floors also covered with wood shavings. The litters were weaned at the age of 21–28 days depending on weight.

c. *Diets*

The basal diets used in all growth and reproduction experiments were similar to those used earlier in rapeseed oil feeding experiments by Roine and Uksila[50] in this laboratory. The semisynthetic basal diet was composed of graham flour, casein,

Table 3.

Compositions of diet types.

Major components	Diet A	Diet B	Diet C	Diet D	Diet E
	%	%	%	%	%
Graham flour	63.4	58.7	44.0	59.0	44.3
Casein	16.4	15.2	11.4	15.3	11.5
Dried brewer's yeast	10.9	10.1	7.6	10.2	7.7
Fat or oil	6.1	13.1	34.6	13.2	34.8
Salt mixture[1]	2.2	2.0	1.5	2.0	1.5
Fat-soluble vitamins in soybean oil[2]	0.9	0.9	0.9	—	—
Codliver oil and vit. E[3]	—	—	—	0.3	0.2
B-vitamins in glucose[4]	0.05	0.05	0.05	—	—
	100	100	100	100.0	100.0

[1] *Salt mixture*

NaCl	1 000 g
Calcium lactate	1 000
Iron citrate	41
$MnSO_4 \cdot H_2O$	23
$CuSO_4 \cdot 5H_2O$	9.4
KI	0.2

[3] *Codliver oil and vitamin E*

Codliver oil	60 g
d,l-α-Tocopherol	1

[2] *Fat-soluble vitamins in 10 ml (9.1 g) soybean oil*

d,l-α-Tocopherol	75.000 mg
Vit. A (20 000 IU)	6.000
Vit. D_3 (1 000 IU)	0.025

[4] *B vitamins in 450 mg glucose*

Calcium pantothenate	20.0 mg
Pyridoxine	20.0
Folic acid	10.0
Biotin	0.6

All the vitamins used were from S.A.F. Hoffman-La Roche & Co.

Table 4.

Energy values of basal diets.

Kilocalories derived from the different foodstuffs per kilogram of diet	Diet A	Diet B	Diet C	Diet D	Diet E
Graham flour	2 066	1 914	1 434	1 923	1 444
Casein	656	608	456	612	460
Dried brewer's yeast	313	290	218	293	221
Fat or oil	539	1 158	3 058	1 167	3 076
Fat-sol. vitamins in soybean oil	80	80	80	—	—
Codliver oil and vit. E	-	—	—	26	18
Vit. B mixture	2	2	2	—	—
	3 656	4 052	5 248	4 021	5 219

dried brewer's yeast, fat or vegetable oil and salt and vitamin mixtures. The exact compositions of the diets are shown in Table 3. The diets were prepared every two weeks and were usually first mixed by hand and then passed through a Wiley laboratory mill or mixed with a Hobart A 200 mixer. They were stored in a cold room (+4—+5°C). The vitamin mixtures as well as the salt mixtures were prepared in larger amounts and the vitamin mixtures were stored in a refrigerator. The nutrient and energy contents of the foodstuffs were calculated according to »Standard Values in Nutrition and Metabolism»,[51] »Die Zusammensetzung der Lebensmittel»[52] and the Food Composition Table of Turpeinen and Roine.[53] The energy values of the different diets were 3.64, 4.03, 5.23, 3.98 and 5.19 kcal per gram (Table 4).

The protein derived from casein amounted to 11—16%, but together with the protein from the graham flour and dried brewer's yeast it accounted for about 20—25% of the total weight of the diet, which usually is considered sufficient,[49,54,55] although McCoy[56] recommends somewhat higher amounts, 25—30%. It is possible that especially during gestation and lactation periods, the animals on the high fat diets suffered from a slight protein deficiency or amino acid imbalance.

Because the primary aim of these experiments was to study the different effects on growth of fats and oil-like substances derived from tall oil fatty acid distillate, the diets contained greater amounts of fat than the usual standard diets for rats, which contain 4—10 wt % fat. The fat or oil to be studied was added to the basal diet at levels of 15, 30 and 60% of the total calories, and the diets were designated according to their fat content and supplement of fat-soluble vitamins. Type A contained 15 cal % fat, type B 30 cal % fat and type C 60 cal % fat and all three types a synthetic fat-soluble vitamin supplement. Types D and E were 30 and 60 cal % diets, respectively, with codliver oil supplement to provide fat-soluble vitamins. When the fat from graham flour and dried brewer's yeast and the small amount of codliver oil or soybean oil which was added with the fat-soluble vitamins are included, the total fat contents were somewhat higher, corresponding to 20, 33 and 61% of the total calories. When butter, margarine and tall oil fatty acid glyceride margarine were used as the fat, their fat content was taken to be 82%.

Of fat-soluble vitamins, only vitamins A and E are considered essential to the rat. The amounts of vitamin A recommended by different authors differ to some extent. McCoy[56] recommends 4 μg of vitamin A or 15 to 20 μg of carotene per kilogram of body weight per day, an anonymous writer in Charles River Digest[54] gives a value of 200—360 IU per day per animal for growth, gestation and lactation and Cuthbertson[55] recommends 3 000 IU per kilogram of food. The fat-soluble vitamins in diets D and E were present in the codliver oil. In view of the recommended amounts of vitamin A, its supply may have been a little too small (Table 5). The need of vitamin E, which seems to be critical especially in reproduction experiments, was well satisfied in all diets, because the recommended amount is 50 mg per kilogram.[57] However, there is some evidence that part of the vitamin E activity was destroyed by oxidation reactions, which easily take place in a codliver oil-vitamin E mixture.[58,59] Apparently vitamin D is not required by the rat if the ratio of calcium and phosphorus is between 1:1 and 2:1 and the amount of phosphorus in the diet is about 0.5%.[56] Because the experimental diets did not meet these requirements, it was considered advisable to add the recommended amounts of vitamin D to diets A, B and C. Vitamin K does not seem to be essential to the rat according to earlier references, but Brüggemann *et al.*[57] recommend addition of 1 mg of menadione per kilogram ration of food.

Water-soluble vitamins of the B group were present in the dried brewer's yeast in amounts that approximately meet the need and even exceed the latter manyfold in the case of thiamine, riboflavin, niacin, pantothenic acid, pyridoxine, vitamin

Table 5.

Nutrients per kilogram of diet and mineral and vitamin needs of the laboratory rat.

	Diet A	Diet B	Diet C	Diet D	Diet E	Estim. need[55]
	g	g	g	g	g	
Protein	283.4	262.1	196.8	263.7	198.2	19–30%
Carbohydrates	485.2	448.7	337.1	450.9	338.9	
Fat	83.8	152.9	364.7	147.9	359.8	
Crude fiber	22.1	20.4	15.3	20.5	15.4	3–5% in synthetic diets
Minerals:	g	g	g	g	g	g/kg diet
Calcium	1.7	1.6	1.2	1.6	1.2	5 –6
Phosphorus	4.4	4.1	3.1	4.1	3.1	3.5–4.0
Potassium	3.4	3.1	2.3	3.1	2.4	5
Sodium	4.2	3.9	2.9	3.9	2.9	5
Chlorine	6.4	5.9	4.4	5.9	4.5	3
	mg	mg	mg	mg	mg	mg/kg diet
Iron	121.3	112.0	84.2	112.7	84.7	50
Copper	30.0	27.6	20.8	27.8	20.9	20
Manganese	43.9	40.5	30.5	40.8	30.6	20
Zinc	17.0	15.7	11.8	15.8	11.9	4
Iodine	1.6	1.5	1.1	1.5	1.1	0.2
Vitamins:						per kg diet
Vitamin A, IU	20 000	20 000	20 000	3 050	2 300	3 000 IU
Vitamin D_3, IU	1 000	1 000	1 000	300	230	1 000 IU
d,l-α-Tocopherol, mg	95.1	93.6	88.9	69.5	52.3	50 mg
Thiamine, mg	16.1	14.9	11.2	15.0	11.3	1–2 mg
Riboflavin, mg	5.2	4.8	3.6	4.8	3.6	3–5 mg
Niacin, mg	80.9	74.8	56.2	75.2	56.5	10 mg
Pantothenic acid, mg	27.9	27.3	25.5	7.3	5.5	10 mg
Pyridoxine, mg	24.8	24.5	23.3	4.5	3.4	1–2 mg
Folic acid, mg	13.5	13.2	12.4	3.2	2.4	—
Biotin, μg	619.7	618.2	613.7	18.3	13.8	—
Vitamin B_{12}, μg	22	20	15	20	15	20–30 μg

B_{12} and choline. In normal situations there seems to be no need for folic acid, biotin, inositol and *p*-aminobenzoic acid,[57] but because of the irregularities which appeared in the reproduction experiments it was considered necessary to add pyridoxine, calcium pantothenate, biotin and folic acid to diets A, B and C in amounts that were twice the amounts of these vitamins in the dried brewer's yeast because oxidized fats have been stated to destroy these vitamins even in the alimentary tract,[60] and a slight deficiency of these vitamins may arise if care is not taken to guarantee a sufficient supply.

The mineral requirements of the rat seem to be supplied by the salt mixture (Table 5), although in some cases the mixture appeared to be slightly insufficient. The calcium amount seems to be lower than the recommended values [55,57] as the ratio of calcium and phosphorus was 1:2.5. The slight calcium deficiency might be one possible cause of some cases of failure in the reproduction experiments and of growth retardation. All trace elements required by the rat seemed to be sufficiently supplied by the salt mixture and other ingredients of the diets.

d. Analytical methods

When the absorbability of a dietary fat was determined, chromium oxide was used as an inert material. Compared with the conventional method in which the food consumed is accurately weighed and the amount and fat content of the feces are determined, the use of an inert material as an indicatior of absorbability makes it possible to collect random samples of feces and the problems connected with food spoilage and leftovers are avoided.

The use of chromium oxide as an inert material in connection with determinations of absorbability was first proposed by Edin[61] as early as 1918 and several workers have demonstrated the applicability of this method in a variety of animal species. Schürch *et al.*[62] evaluated the use of chromium oxide as an indicator in the rat and found that identical results for absorbability were obtained when random samples taken at various times of the day were analyzed.

In the present determinations of the absorbability of the tall oil fatty acid distillate and its ethyl and glyceryl esters, 0.5% chromium (III) oxide (Noury & Baker, N.V., lab. grade) was added to the diet. When the chromium content in the feces had reached a constant level, which usually took four or five days, the feces were collected from each rat separately during 5–20 days. The feces were stored under nitrogen at –20°C to avoid oxidation, because the fatty acid composition of the fecal fat was determined in addition to the digestibility coefficient.

All the other chemicals employed in the work were guaranteed reagents from E. Merck AG or AnalaR reagents from The British Drug Houses Ltd. The chromium content was determined by a method described by Paloheimo and Paloheimo.[63]

For the chromium determinations, 250 mg of vacuum-dried, powdered fecal matter was weighed in a crucible and ashed at 700°C for 2.5 hours. Five grams of sodium peroxide was added to the finely ground ash and the crucible was reheated at 700°C for 10 min. The sodium chromate which had formed was transferred from the crucible to a beaker with 200 ml of boiled water in small portions. After the effervescence had stopped, the liquid was partly neutralized with 20 ml of 3 N hydrochloric acid, diluted to one liter and left to stand for about 30 minutes. The absorbance was measured with a Beckmann DU spectrophotometer at 430 nm and the chromium content was read from a standard curve. Duplicate determinations were performed on each sample.

In other experiments the chromium oxide content was determined with an atomic absorption spectrophotometer according to Williams *et al.*[64] The ashed sample was dissolved in 3 ml of a phosphoric acid-manganese sulphate solution (30 ml of 10% w/v $MnSO_4 \cdot 4H_2O$ solution in one liter of 85% phosphoric acid) and oxidized with 4 ml of 4.5% potassium bromate. After digestion on a hot plate the sample was cooled diluted with water and transferred quantitatively to a 200-ml volumetric flask. Twenty-five milliliters of a calcium chloride solution containing 4 000 ppm of calcium was added to correct for calcium interferences and to suppress silicate and aluminum interferences, since Williams *et al.* have shown that in the presence of more than 500 ppm of calcium, the chromium absorption changes very little and that magnesium does not affect the absorption. A portion of the sample to be analyzed was filtered to remove suspended material and a subsample was analyzed on a Perkin Elmer model 303 atomic absorption spectrophotometer. A chromium hollow cathode lamp was used and the readings were made at the chromium resonance line at 358 nm. Blank and standard solutions were prepared from potassium dichromate and the chromium content was read from the standard curve.

The fat content was determined by a method described by King[65] on the same samples as the chromium content. The fecal sample (usually 3–5 g) was dried in a vacuum oven overnight at 60°C and ground to a fine powder in a mortar. Five hundred milligrams of the sample was weighed in a glass-stoppered 65-ml Pyrex test tube, 10 ml of distilled water was added and the fatty acids were liberated from their soaps with 3 ml of concd. hydrochloric acid. The tube was heated for 10 min in a boiling water bath and then cooled. The fatty acids were extracted by adding 50 ml of petroleum ether and shaking the tube vigorously for 10 min. The layers were allowed to separate completely and 25 ml of the petroleum ether layer was pipetted into a Soxhlet flask through a filter paper covered by a layer of anhydrous sodium sulphate. The filter paper and the sodium sulphate layer were washed with 25 ml of petroleum ether and the washings passed into the flask. The flask was connected to a Soxhlet apparatus and the petroleum ether evaporated on a water bath (80°C). The flask was then dried in a vacuum oven at 60°C for one hour and weighed. Duplicate determinations were made on every sample.

The digestibility coefficient of the fat consumed was calculated from the formula

$$100\left(\frac{a-b}{a}\right),$$

where a = amount of fat per gram of chromium oxide in the food
b = amount of fat per gram of chromium oxide in the feces

The conventional method of weighing the food consumed and the feces collected during a certain period was employed to see how well the results corresponded to the results of the chromium oxide method applied to feces of the same rats immediately afterwards. The differences, only 0.2–0.5%, were not significant.

2. Results

a. Growth experiments

Growth is most commonly used as a criterion when assessing the need of a nutritional factor in the diet of an animal, because growth imposes a great metabolic strain on the animal. Retarded growth is considered a symptom of a disease or a disturbance of the function of the organism. It is possible to find out within a relatively short period the growth-promoting or growth-retarding characteristics of a certain substance compared with another, well-known substance.

In this study, as stated before, the effects of tall oil fatty acid distillate and its ethyl and glyceryl esters on the growth of rats were studied. The control animals received equal amounts of soybean oil, butter or margarine. The experimental conditions were otherwise the same for the experimental and control animals.

The short-term experiments usually lasted from two to four weeks. Some of them were only orientative experiments. Only male rats were used in these experiments because they grow much more rapidly than female rats. The long-term experiments lasted two to three times longer than the short-term experiments and both male and female rats were used.

Tall oil fatty acid distillate

The aim of this experiment was to investigate the effect of tall oil fatty acid distillate as such on the growth of rats. The basal diet types B and C were used and the tall oil fatty acid distillate was added at levels of 30 and 60% of the total calories. In addition, a group receiving 15% of the total calories as tall oil fatty acid distillate (diet A) was included, because higher fat levels proved to be lethal. The control groups received the same amounts of soybean oil.

The type and amount of fat in the diets, the average initial weights of the groups, their average cumulative weekly weight gains, the total number of animals, the number of animals that died and the average food consumptions during the experimental period are presented in Table 6. The cu-

Table 6.

Effect of tall oil fatty acid distillate on the weight gain of young rats.

Type and amount of fat in the diet		Mean initial weight, g	Average cumulative weight gain, g Week of experiment 1.	2.	3.	4.	Deaths	Average food consump- tion,[1] g
Tall oil fatty acid distillate	15 cal %	40.3	16.2	39.4	63.6	95.5	1/10	274.7
Tall oil fatty acid distillate	30 »	40.1	4.6	16.6	35.9	55.3	0/10	170.5
Tall oil fatty acid distillate	60 »	39.9	—[1]	—	—	—	10/10	—
Soybean oil	15 »	39.8	23.0	50.5	80.3	101.5	0/10	316.3
Soybean oil	30 »	40.0	30.0	59.6	98.9	128.1	0/10	313.6
Soybean oil	60 »	40.1	17.9	35.3	57.6	85.1	0/10	205.6

[1] All animals died within 4 days.

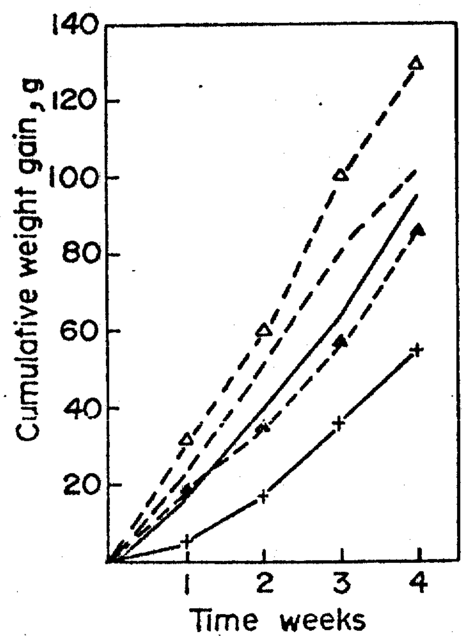

Fig. 1. Cumulative growth curves for rats fed tall oil fatty acid distillate and soybean oil fatty acids.

———————	15 cal % tall oil fatty acid distillate
—×—×—×—	30 » » » » » »
— — — — — —	15 » soybean oil fatty acids
—△—△—△—	30 » » » » »
—▲—▲—▲—	60 » » » » »

mulative weight gains of the groups are plotted against time in weeks in Fig. 1, from which it can be seen that when 15% of total calories were derived from tall oil fatty acid distillate the growth rate of the rats was almost equal to that of rats receiving soybean oil at levels of 15 and 60% of the total calories. The group receiving 30% of total calories as tall oil fatty acid distillate grew slowest. This seems to be a consequence of the weak appetite this group had; the average total food consumption of 170 g during a period of 4 weeks was only slightly more than half the amount eaten by the group receiving soybean oil at a level of 15% in the diet. The group receiving 30% of total calories as soybean oil grew more rapidly despite a smaller food consumption. One death occurred in the group receiving 15% of total calories as tall oil fatty acid distillate, but no deaths occurred at the 30% level. All animals in the group receiving 60% of total calories as tall oil fatty acid distillate died within a few days. There is a statistically highly significant difference ($P < 0.001$)* in the final weight between the group receiving 30% of total calories as tall oil fatty acid distillate and the control group given soybean oil at the same caloric level. The difference at the lower fat level is not significant. The most noticeable feature of this experiment is the death of the whole group receiving 60% of total calories as tall oil fatty acid distillate. It was concluded that there is a growth-retarding or possibly a toxic factor in the tall oil fatty acid distillate.

Pine seed oil

In order to determine the role of *cis*-5,9,12-octadecatrienoic acid as a possible growth-retarding factor, two short experiments were conducted using pine seed oil as the fat ingredient in the diet. Pine seed oil contains twice as much *cis*-5,9,12-octadecatrienoic acid as tall oil fatty acid distillate. The results of these experiments are shown in Table 7, and the growth curves are presented in Fig. 2.

The basal diet used in these experiments was type D. The control groups in the first experiment received either ordinary soybean oil or soybean extract isolated in the same way as pine seed oil. In the second experiment only the soybean oil was given to the control group. The animals

Table 7.

Effect of pine seed oil on weight gain of young rats.

Type and amount of fat in the diet	Mean initial weight, g	Average cumulative weight gain, g							Deaths	Average food consumption, g
		Day of experiment								
Experiment I		2.	5.	7.	10.	12.	14.	16.		
Pine seed oil 30 cal %	47.0	7.9	21.2	29.3	44.1	54.7	63.7	73.1	0/10	167.2
Soybean extract 30 »	46.8	9.3	25.8	36.4	52.5	63.7	73.4	82.8	0/10	181.9
Soybean oil 30 »	47.2	8.4	23.1	33.0	49.0	60.8	71.7	82.2	0/10	181.1
		Day of experiment								
Experiment II		2.	4.	6.	8.	10.	13.			
Pine seed oil 30 »	47.0	7.0	12.5	21.0	31.5	41.5	56.5		0/2	117.7
Soybean oil 30 »	45.5	8.0	13.0	21.0	30.5	40.5	57.0		0/2	114.5

* The statistical significances were determined by Student's t-test. Tables of LINDLEY and MILLER[66] were used.

132

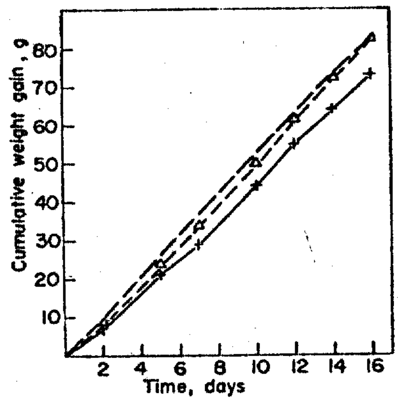

Fig. 2. Cumulative growth curves for rats fed pine seed oil, soybean oil and soybean extract.

—x—x—x—	30 cal %	pine seed oil
—△—△—△—	30 »	soybean oil
— — — —	30 »	soybean extract

receiving pine seed oil in their diets grew at a slightly lower rate than the control animals in the first experiment and their food consumption was somewhat smaller than that of the control groups, but no statistically significant differences were found. In the second experiment there were only two animals per group because the pine seed oil was difficult to obtain. Therefore the material is too small to draw conclusions, but it appears that both groups gained equally in weight and that there was only a slight difference in average food consumption.

These results seem to indicate that *cis*-5,9,12-octadecatrienoic acid is not the growth-retarding component of the tall oil fatty acid distillate, but that the toxic component is more likely produced in the distillation of tall oil. Unfortunately, the amount of pine seed oil available was so small that no experiments could be made with a higher level of the oil in the diet.

Ethyl esters of distilled tall oil fatty acids

The product made from tall oil fatty acids which has been used in earlier experiments with cattle and poultry is the mixture of ethyl esters of distilled tall oil fatty acids. Several experiments in which esterified tall oil fatty acids were added to the diet were also performed with rats in this study. Both young and adult animals were used and the amounts of ethyl esters of tall oil fatty acids given amounted to 30 and 60% of the total calories. The basal diets used in these experiments were types B and C. As seen from the results of an experiment with young animals shown in Table 8, the toxic effect of the higher fat level seems to be almost as strong as that of the unesterified tall oil fatty acid distillate. The consumption of food containing the higher fat level was very small in this group as compared with the soybean oil group. A statistically highly significant difference ($P < 0.001$) was found between the mean weights of the test and control groups. As seen from the same table, experiments with adult animals gave better results. Even here, however, only the animals receiving the smaller amount of ethyl esters of tall oil fatty acids gained weight but relatively more slowly than the animals in the control group receiving soybean oil. The group which received 60% of total calories as ethyl esters of tall oil fatty acids suffered a weight loss of 18.5% during the experimental period of almost 4 weeks. In this experiment, too, a statistically highly significant difference ($P < 0.001$) was found between the test and control groups at the 60% fat levels and also between the groups receiving ethyl esters of tall oil fatty acids at the 30 and 60% levels. On the lower fat level the difference between the test and control groups was significant ($P < 0.01$). It is possible that the results for the group on the higher fat level might have been even worse after a longer experimental period, since skin lesions

Table 8.

Effect of ethyl esters of tall oil fatty acid distillate on weight gain of rats.

Type and amount of fat in the diet		Mean initial weight, g	Average cumulative weight gain, g						Deaths	Average food consumption, g
			Day of experiment							
Young rats			2.	5.	6.	8.	10.	12.		(10 days)
Ethyl esters of tall oil fatty acids	60 cal %	48.7	−2.5	−5.0	−6.4	−7.0	−7.0	−3.2	6/10	39.0
Soybean oil	60 »	48.4	4.8	12.9	15.8	24.5	32.2	40.3	0/10	79.4
			Week of experiment							
Adult rats			1.	2.	3.	4.				(2 weeks)
Ethyl esters of tall oil fatty acids	30 »	229.2	0.8	16.7	8.4	26.9			0/10	225.2
Ethyl esters of tall oil fatty acids	60 »	229.7	−31.6	−36.4	−53.0	−42.5			1/10	107.9
Soybean oil	30 »	229.4	15.0	48.2	51.8	74.9			0/10	258.8
Soybean oil	60 »	229.8	12.1	40.9	60.9	69.8			0/10	117.8

were observed and the animals had glossless, shaggy fur. It seems that the growth-retarding factor has a strong effect even when present in esterified tall oil fatty acids.

Glyceryl esters of distilled tall oil fatty acids

The tall oil fatty acid product which most closely resembles ordinary vegetable oils, such as soybean oil, is the mixture of glyceryl esters of distilled tall oil fatty acids. It was therefore thought that this synthetic oil might be better for feeding purposes than the mixture of ethyl esters of tall oil fatty acids. The types of diet used in the experiments were D and E, and the test fat was either the mixture of glyceryl esters of tall oil fatty acids or a clarified product derived from the glyceryl ester mixture by cooling the latter to +4°C and centrifuging to isolate the clear layer. Both the clarified mixture and the mixture of original glyceryl esters of tall oil fatty acids were used at levels of 30 and 60% of the total calories. The results of the growth experiments are presented in Table 9. It can be seen that both test groups receiving either the unclarified or the clarified glyceryl esters of tall oil fatty acids grew equally well, but they lagged much behind the control group that received 30% of the calories as soybean oil. The differences are statistically highly significant ($P < 0.001$). The food consumptions of both groups receiving glyceryl esters of tall oil fatty acids were almost equal but less than the food consumption of the soybean oil group. At the higher fat level, the differences in growth rate between the control group receiving soybean oil and the groups receiving the original and clarified glyceryl esters of tall oil fatty acids were even more significant, because the latter animals lost weight during the first half of the experiment and only very slowly approached the original level. Five and six of the ten animals in each of the two groups receiving the higher level of glyceryl esters of tall oil fatty acids died during the experimental period of 20 days. There were highly significant differences ($P < 0.001$) between the control group and the groups receiving the higher level of either clarified or ordinary glyceryl esters of tall oil fatty acids, and also between the groups receiving the lower and higher levels of the glyceryl esters of tall oil fatty acids.

Different fractions of ethyl and glyceryl esters of the distilled tall oil fatty acids

1. Fractionated tall oil fatty acids

The distilled fatty acid fraction of tall oil contains, as mentioned earlier, a small amount, about 2%, of resin acids. After esterification, some unsaponifiable matter, usually less than 2%, remains in the final ester mixture.

Table 9.

Effect of glyceryl esters of distilled tall oil fatty acids on weight gain of young rats.

Type and amount of fat in diet		Mean initial weight, g	Average cumulative weight gain, g Day of experiment								Deaths	Average food consumption, g
			2.	5.	7.	9.	13.	15.	17.	20.		
Glyceryl esters of tall oil fatty acids	30 cal %	51.6	−2.8	5.0	9.5	14.5	24.2	27.1	31.9	43.3	1/10	132.2
Glyceryl esters of tall oil fatty acids	60 »	51.6	−7.9	−7.1	−6.2	−6.0	−5.2	−4.5	−4.5	−1.8	5/10	61.3
Clarified glyceryl esters of tall oil fatty acids	30 »	51.7	−2.1	5.2	9.6	12.4	19.6	26.5	30.5	44.7	2/10	123.9
Clarified glyceryl esters of tall oil fatty acids	60 »	51.7	−7.5	−7.3	−6.8	−6.4	−2.5	1.1	−0.3	3.3	6/10	54.5
Soybean oil	30 »	51.5	3.7	17.9	26.7	33.4	54.1	61.9	70.8	86.1	1/10	186.8
Soybean oil	60 »	51.6	1.3	11.3	17.1	21.4	35.5	41.0	47.4	58.4	0/10	127.0

The possibility that either of these fractions may have a detrimental effect on the growth of animals was studied by mixing 5 parts of either the resin acids or the unsaponifiable matter with 95 parts of soybean oil and adding these fat mixtures to the diets of young animals at a level of 60% of total calories. In the same experiment, diets which contained 10 parts of either glyceryl esters of tall oil fatty acids or the elaidin precipitate derived from it and 90 parts of soybean oil at a level of 60% of total calories were fed. The control group received soybean oil as before and one additional group was included that received glyceryl esters of tall oil fatty acids, both at the higher level. The basal diet used was E. The results, which are presented in Table 10, indicate that the best growth was obtained with soybean oil. The weight gain was somewhat lower when glyceryl esters of tall oil fatty acids or the elaidin precipitate derived from it was given with soybean oil, but higher than the weight gain of the groups receiving resin acids or unsaponifiable matter in their diets, though the differences were not very marked. When all the fat included in the diet was a mixture of glyceryl esters of tall oil fatty acids, the growth-retarding effect was obvious, and the result was comparable with that of the earlier experiment where glyceryl esters of tall oil fatty acids were fed at a level of 60% of total calories. Statistical calculations revealed that there was a highly significant difference ($P < 0.001$) between the weight gain of the control group and that of the group receiving 60% of total calories as glyceryl esters of tall oil fatty acids. A significant difference ($P < 0.01$) was found between the weight gains of the control group and the group receiving 5% of the total diet fat as resin acids. The food consumption of the control group was almost twice as high as that of the group receiving 60% of total calories as glyceryl esters of tall oil fatty acids. The group receiving 10% of the total fat as the elaidin precipitate derived from the glyceryl esters of tall oil fatty acids consumed almost as much food as the control group, but the groups receiving the unsaponifiable matter and the resin acid fraction in their diet consumed somewhat less food. The toxic nature of the glyceryl esters of tall oil fatty acids became clearly evident also in this experiment, because two thirds of the animals receiving the mixture in their diet died during the experiment. Two of the six animals in the group receiving the unsaponifiable fraction and one of the animals of the resin acid group died during the experiment.

2. Unheated and heated glycerol

The effect of the glycerol used in the esterification of tall oil fatty acids was studied in a separate experiment. The control group received 60% of total calories as soybean oil. Two test groups, one of them receiving

Table 10.

Effect of different fractions of glyceryl esters of distilled tall oil fatty acids on weight gain of young rats.

Type and amount of fat in diet		Mean initial weight, g	Average cumulative weight gain, g — Day of experiment 2.	5.	7.	10.	12.	14.	19.	21.	Deaths	Average food consumption, g
Glyceryl esters of tall oil fatty acids	60 cal %	49.1	−3.6	−6.1	−7.1	−5.4	−5.1	6.9	10.9	9.9	4/6	74.5
Soybean oil/glyceryl esters of tall oil fatty acids 90:10	60 »	49.1	4.5	13.2	19.9	28.2	33.2	40.7	50.7	58.5	0/6	120.0
Soybean oil/glyceryl esters of elaidin precipitate 90:10	60 »	49.3	4.8	9.7	18.5	26.3	31.7	39.3	55.5	65.2	0/6	133.4
Soybean oil/unsaponifiable matter of tall oil fatty acid distillate 95:5	60 »	49.3	5.3	11.8	19.5	23.0	27.5	29.7	47.4	53.9	2/6	119.5
Soybean oil/rosin acids of tall oil fatty acid distillate 95:5	60 »	49.3	−0.8	2.0	8.0	19.7	24.9	29.9	45.9	54.1	1/6	109.3
Soybean oil	60 »	49.3	3.5	13.5	21.8	30.5	38.7	44.9	64.5	72.9	0/6	134.1

132

Table 11.

The effect of glycerol on weight gain of young rats.

Type and amount of fat in the diet		Mean initial weight, g	Average cumulative weight gain, g — Day of experiment 2.	5.	7.	10.	12.	17.	19.	21.	Deaths	Average food consump- tion, g
Glyceryl esters of tall oil fatty acids	60 cal %	46.4	−1.5	−0.4	1.2	3.7	6.9	11.0	10.0	8.8	5/10	74.9
Soybean oil	60 »	46.5	4.8	11.6	15.9	24.4	29.7	52.8	56.6	62.8	1/10	138.3
Soybean oil/glycerol 90:10	60 »	46.4	7.4	14.1	22.2	33.3	37.9	55.5	62.2	71.5	1/10	158.9
Soybean oil/heated glycerol 90:10	60 »	46.9	7.6	15.5	21.6	29.8	35.3	50.2	54.1	61.9	1/10	137.1

Table 12.

The effect of ethyl esters *cis*-5,9,12-octadecatrienoic acid on weight gain of young rats.

Type and amount of fat in the diet		Mean initial weight, g	Average cumulative weight gain, g — Day of experiment 2.	4.	7.	10.	15.	19.	23.	25.	Deaths	Average food consump- tion, g
Ethyl esters of tall oil fatty acids	30 cal %	83.8	1.1	7.7	19.7	33.2	49.5	59.3	73.0	80.9	0/10	219.4
Ethyl esters of 5,9,12-octadecatrienoic acid	30 »	81.3	−6.3	−4.3	0	2.8	5.9	10.6	16.4	19.3	0/10	157.5
Ethyl esters of linseed oil fatty acids	30 »	84.4	5.4	15.8	30.2	43.9	65.9	77.2	96.6	106.4	0/10	251.7

10% of the total fat as glycerol mixed with 90% of soybean oil and the other receiving the same amount of glycerol which had been heated to 200°C, were included in this experiment. In addition, one group received glyceryl esters of tall oil fatty acids at the 60% level. The basal diet was type E. The results are summarized in Table 11. It will be seen that neither the unheated nor the heated glycerol had any essential effect on the growth, for the weight gains in the control group and both groups receiving glycerol together with soybean oil were almost equal. Also the food consumptions were almost equal and one death occurred in each of these groups during the experimental period. However, a difference became evident in this experiment between the control group and the group receiving glyceryl esters of tall oil fatty acids; the gain in weight was about seven times larger and the food consumption was about twice as high in the control group. Half of the animals receiving the mixture of glyceryl esters of tall oil fatty acids died during this experiment. The difference between the average weights of the control group and the survivors of the group receiving glyceryl esters of tall oil fatty acids at the end of the experiment was statistically highly significant ($P < 0.001$).

3. The concentrate of *cis*-5,9,12-octadecatrienoic acid

The locations of the double bonds in *cis*-5,9,12-octadecatrienoic acid are uncommon in natural fats and it was at first suspected that this exceptional acid might be one factor responsible for the growth-retarding and toxic effects of the tall oil fatty acid distillate. Later experiments, especially the one in which pine seed oil was the diet fat, partly disproved this theory, but the results of one experiment in which the ethyl esters of an 80% concentrate of the *cis*-5,9,12-octadectrienoic acid was given may be of interest.

The ethyl esters of the concentrate of the *cis*-5,9,12- octadecatrienoic acid were fed at a level of 30% of total calories and the control groups received the same amount of either ethyl esters of tall oil fatty acids or ethyl esters of linseed oil fatty acids; the latter was chosen because of the high content of ethyl linolenate. The basal diet was D. The results are presented in Table 12. It will be seen that the weight gain was clearly smaller and the food consumption much lower in the group receiving the ethyl ester of the *cis*-5,9,12-octadecatrienoic acid concentrate than in the other groups. Statistical analysis confirmed that there were highly significant differences between the weight gains of the groups receiving either ethyl esters of linseed oil fatty acids or ethyl esters of tall oil fatty acids and the group receiving the ethyl ester of the *cis*-5,9,12-octadecatrienoic acid concentrate ($P < 0.001$).

4. Fractions of tall oil fatty acids separated with urea.

By fractionation of tall oil fatty acids with urea, four fractions were obtained which were esterified with ethanol. A short experiment, lasting only 10 days, was carried out using these esterified fractions and ethyl esters of tall oil fatty acids in the diets at a level of 60% of total calories. The control group received ethyl esters of soybean oil fatty acids and the basal diet used was of type E. The results, which are presented in Table 13, show that the only fraction which seemed to promote growth more than the others was fraction 1. Fractions 2 and 3 were almost equal in their effects to the ethyl esters of tall oil fatty acids; the weights of the rats in these groups dropped continuously and half to two thirds of the animals died during the short experimental period. Fraction 4, which contained most of the *cis*-5,9,12-octadecatrienoic acid, seemed to be highly toxic, since all the animals of this group died within three days. There was a statistically significant difference between the weight gains of the control group and the group receiving fraction 1 ($P < 0.01$) and highly significant differences between the control group and the groups receiving fraction 2, fraction 3 or the ethyl esters of tall oil fatty acids ($P < 0.001$). The food intake was not recorded in these experiments.

5. Molecularly distilled glycerides of tall oil fatty acids

Molecular distillation of the glycerides of tall oil fatty acids was undertaken to determine whether the content of unsaponifiable matter in the glyceride mixture can be reduced by molecular distillation.[46] Six different distillate fractions were collected. The first two fractions contained most of the unsaponifiable matter. Fractions 3—6 (distilled in the temperature

Table 13.

The effect of ethyl esters of fractions of tall oil fatty acid distillate separated with urea on weight gain of young rats.

Type and amount of fat in the diet		Mean initial weight, g	Average cumulative weight gain, g					Deaths
			Day of experiment					
			2.	4.	6.	8.	10.	
Ethyl esters of tall oil fatty acids	60 cal %	48.8	−4.1	−0.3	−3.8	−4.3	−5.8	0/8
Esterified tall oil fatty acid fraction 1	60 »	48.8	0.1	3.6	7.1	11.9	17.5	1/8
Esterified tall oil fatty acid fraction 2	60 »	48.8	−5.2	−3.2	−3.5	−3.8	−5.0	4/8
Esterified tall oil fatty acid fraction 3	60 »	48.8	−6.5	−5.3	−4.3	−4.8	−7.3	6/8
Esterified tall oil fatty acid fraction 4	60 »	48.6	−10.2	—[1]	—	—	—	8/8
Ethyl esters of soybean oil » »	60 »	48.6	3.3	11.8	17.7	25.7	35.4	0/8

[1] All animals died within 3 days.

Table 14.

The effect of the molecular distillate of the glyceryl esters of distilled tall oil fatty acids on weight gain of young rats.

Type and amount of fat in the diet		Mean initial weight, g	Average cumulative weight gain, g — Day of experiment 2.	4.	7.	10.	12.	14.	18.	21.	Deaths	Average food consumption, g
Molecular distillate of glyceryl esters of tall oil fatty acids	60 cal %	61.5	−3.4	−5.0	−4.9	−1.9	−2.6	−1.4	1.7	2.3	1/10	90.6
Soybean oil	60 »	61.5	3.2	6.6	13.5	24.0	28.3	37.2	48.3	63.1	0/10	156.1

Table 15.

The effect of hydrogenated glyceryl esters of distilled tall oil fatty acids on weight gain of young rats.

Type and amount of fat in the diet		Mean initial weight, g	Average cumulative weight gain, g — Day of experiment 2.	4.	6.	10.	13.	15.	17.	21.	23.	Deaths	Average food consumption, g
Glyceryl esters of tall oil fatty acids	30 cal %	62.7	2.6	7.4	11.6	22.5	31.7	35.6	40.9	57.6	63.6	0/10	196.5
Glyceryl esters of tall oil fatty acids	60 »	62.7	−4.9	−6.4	−7.0	−5.9	−6.3	−9.7	−10.0	−7.1	−5.6	4/10	93.0
Soybean oil	30 »	62.6	8.9	15.7	20.8	31.3	39.9	46.5	54.5	71.1	79.5	1/10	226.2
Soybean oil	60 »	62.7	6.9	11.3	13.7	21.6	36.2	46.4	52.1	71.6	77.4	2/10	178.6
Hydrogenated tall oil fatty acid glycerides	30 »	62.7	5.8	12.7	17.8	28.2	38.6	45.7	51.1	70.8	76.8	0/10	228.1
Hydrogenated soybean oil	30 »	62.6	6.4	13.6	19.7	31.1	44.6	51.0	55.6	75.6	79.5	0/10	247.4

range 200—260°C) were combined and fed to the rats at a level of 60% of total calories. In order to assure an adequate amount of essential fatty acids, 5 wt % of the total fat was soybean oil. The control group received soybean oil. The basal diet was E. The results of this experiment (Table 14) indicate that the growth rate of the experimental group did not differ essentially from the growth rates in the earlier experiments where ethyl or glyceryl esters of the tall oil fatty acids were fed. The experimental group lost weight initially and the weight did not rise to the original level before the end of the experiment. Only one animal of this group died during the experiment, but a statistically highly significant difference ($P < 0.001$) was found between the weight gains of the control group and the experimental group.

Hydrogenated glycerides of distilled tall oil fatty acids

When the glyceryl esters of the tall oil fatty acids were hydrogenated, a substance was obtained which melted at 37°C and which after refining and bleaching closely resembled food fats in many respects including the chemical and physical constants. The fat was used in feeding experiments at a level of 30% of the total calories. The other fats which were used in the same experiments were soybean oil and glycerides of tall oil fatty acids at levels of 30 and 60% and hydrogenated soybean oil (mp 32°C) at a level of 30% of total calories. The basal diets used were D and E. The results of this experiment are presented in Table 15. The favorable effect of hydrogenation on the glycerides of tall oil fatty acids is clearly seen; the weight gain of this group is almost equal to the weight gains of the control groups receiving either soybean oil or hydrogenated soybean oil. The group receiving 30% of total calories as glycerides of tall oil fatty acids did not gain as much weight as the control groups, although the differences were not statistically significant. On the other hand, the group receiving glycerides of tall oil fatty acids at the higher level lost weight and the animals did not regain their initial weights during the experiment. The difference in the weight gain of the control group given 60% of total calories as soybean oil and the group given the same level of glycerides of tall oil fatty acids was highly significant ($P<0.001$).

Margarine prepared from hydrogenated tall oil fatty acid glycerides

The experiments in which margarine prepared from hydrogenated tall oil fatty acid glycerides was fed lasted from five to ten weeks during which time the weanling rats grew up to adults. A longer experimental period gives better possibilities of following the development and well-being of the animals than a shorter period when most observations are made on the growth of a certain group compared with a control group. A long-term experiment can be followed by a reproduction experiment, which may give further information on the physiological effects of the food components. Usually larger groups are required in such experiments than in a shorter experiment. The aim in these long-term experiments was to have groups of about 15–20 animals, both males and females, but some of the groups were smaller.

These studies included a series of experiments where three successive generations of rats were fed the tall oil fatty acid glyceride margarine. The tall oil fatty acid glyceride margarine resembled ordinary margarine in appearance and odor, but differed slightly in taste.

The first experiment (Experiment I) of this series was carried out using tall oil fatty acid glyceride margarine and, as control fats, butter and margarine at 30% and 60% levels of total calories. The water contents of these fat products were taken into account by assuming that the fat contents were 82%. The basal diets were D and E. Data on the number of animals, weight gains, the experimental period, etc., are presented in Table 16.

Of the males, the control group that received 30% of total calories as butter had the highest weight gain as is seen in Fig. 3, but the weight gain of the experimental group receiving 30% of total calories as tall oil fatty acid glyceride margarine was almost as high and exceeded that of the ordinary margarine group at the end of the experiment. All groups receiving 60% of their calories as butter, margarine or tall oil fatty acid glyceride margarine grew at lower rates but the differences between the control groups and the experimental group were insignificant. There were no statistically significant differences between the weight gains of the control groups and the experimental groups at either level, but when the experimental groups at the lower and higher levels were compared the difference was statistically highly significant ($P < 0.001$). The food consumptions of the different groups agreed with the growth rates. There were some deaths in the different groups, but the mortality was not greater in the high fat level groups than in the low fat level groups.

The growth curves of the females were similar to those of the males, but their growth rates were much lower. The highest gain in weight occurred in the group receiving 30% of total calories as tall oil fatty acid glyceride margarine. The group receiving butter at the same level did not lag much behind, but the group receiving 60% of total calories as margarine passed the group receiving the lower amount of margarine. Also in this experiment the group receiving 60% of total calories as tall oil fatty acid glyceride margarine grew at the lowest rate. Statistical calculations indicated that there were no significant differences between the control groups and the

Table 16.

The effect of tall oil fatty acid glyceride margarine on weight gain of rats. Experiment I.

Type and amount of fat in the diet		Mean initial weight, g	Average cumulative weight gain, g — Week of experiment 1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	Deaths	Average food consumption, g
Males														
Tall oil fatty acid glyceride margarine	30 cal %	42.5	37.3	64.7	86.1	108.1	134.8	152.8	163.0	199.2	216.0	223.3	2/15	897.7
Tall oil fatty acid glyceride margarine	60 »	42.2	29.8	51.0	71.4	90.8	121.0	143.8	157.8	169.8	189.0	195.8	0/5	711.2
Butter	30 »	42.5	38.8	68.8	94.9	116.2	150.4	175.4	190.7	209.2	226.6	233.3	1/15	900.0
Butter	60 »	42.2	30.8	51.2	74.6	93.8	128.8	148.2	163.2	178.4	197.4	202.4	0/5	682.2
Margarine	30 »	42.5	39.4	64.6	90.6	109.0	141.0	157.0	172.2	139.0	202.6	211.2	2/15	887.1
Margarine	60 »	42.2	23.2	50.6	63.0	95.1	121.1	144.3	159.3	134.3	200.8	207.5	2/5	703.0
Females														
Tall oil fatty acid glyceride margarine	30 »	46.0	27.7	50.6	67.8	80.4	99.1	111.7	118.8	126.1	134.6	135.9	0/19	754.4
Tall oil fatty acid glyceride margarine	60 »	45.9	16.2	37.5	51.7	65.2	80.6	94.1	101.1	107.8	117.1	122.0	0/10	525.0
Butter	30 »	46.2	29.2	48.4	63.1	75.9	91.3	105.5	113.7	121.5	130.7	134.7	0/19	766.8
Butter	60 »	45.8	22.2	43.4	59.7	72.7	90.0	102.9	109.8	116.1	122.1	125.1	0/10	619.0
Margarine	30 »	46.1	27.8	47.3	65.7	76.6	93.8	104.2	113.2	121.0	128.9	130.7	1/19	752.0
Margarine	60 »	45.8	21.1	44.9	61.6	76.8	92.3	102.0	108.0	118.0	125.4	132.2	1/10	604.2

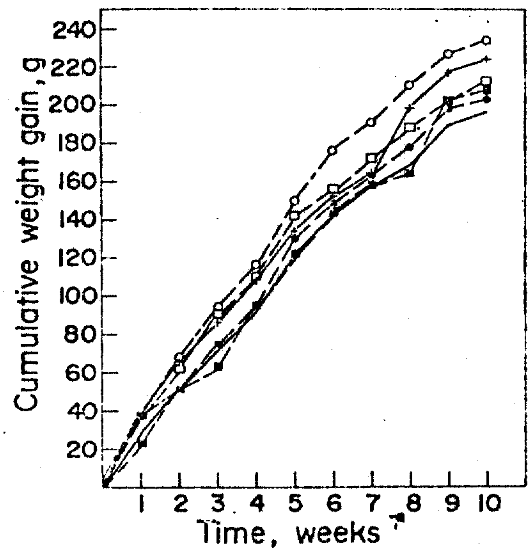

Fig. 3. Cumulative growth curves for male rats fed tall oil fatty acid glyceride margarine, butter and margarine. I generation.

—x—x—x—	30	cal %	tall oil fatty acid glyceride margarine
————————	60	»	» » » » » »
—o—o—o—	30	»	butter
—●—●—●—	60	»	»
□—□—□—	30	»	margarine
—■—■—■—	30	»	» »

experimental group at the lower fat level. The situation was the same at the 60% level but a probably significant difference existed between the weight gains of the experimental groups receiving the lower and higher fat levels ($P < 0.05$). Only two deaths occurred in the female groups, one in each of the groups receiving ordinary margarine.

Long-term experiment II was carried out with the offspring of the first generation. The general features of this experiment were like those of the previous one, but a few alterations were made in the diet. In connection with the reproduction experiments to be described below, »ring-tail» disease was observed in the litters, and one cause of the disease was assumed to be the low content of essential fatty acids compared to the considerably higher content of saturated fatty acids in the diet fat. For this reason, 20 grams of soybean oil was added per kilogram of the diets D and E. At the lower fat level, the fat consisted of 16% soybean oil and 84% butter, margarine or tall oil fatty acid glyceride margarine, but at the higher fat level the proportion of soybean oil was only 4.5% and this was mixed with 95.5% of the experimental or control fats. According to Holman,[67] a

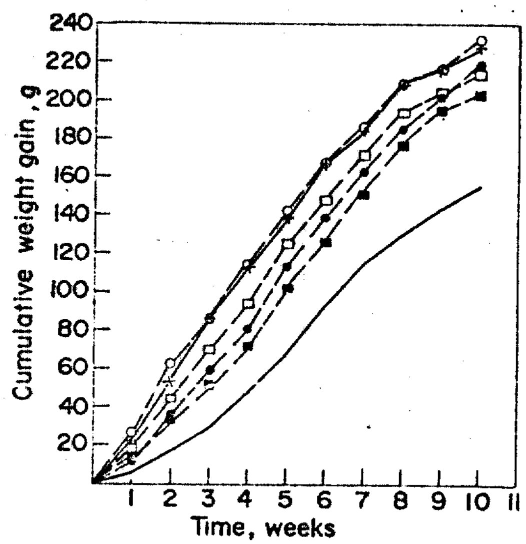

Fig. 4. Cumulative growth curves for male rats fed tall oil fatty acid glyceride margarine, butter and margarine. II generation.

—x—x—x—	30 cal %	tall oil fatty acid glyceride margarine
————	60 »	» » » » »
—o—o—o—	30 »	butter
—●—●—●—	60 »	»
—□—□—□—	30 »	margarine
—■—■—■—	60 »	»

rat's requirement of essential fatty acids as linoleate is approximately 2% of the total calories. Twenty grams of soybean oil per kilogram of diet gives more than 10 grams of linoleic and linolenic acids per kilogram of diet and together with the essential fatty acids in the other fats, the diets contained sufficient essential fatty acids.

The results presented in Table 17 agree with those of the first tall oil fatty acid glyceride margarine experiment. The initial average weights of the different groups differed slightly because only a limited number of offspring of the first generation was available. The highest gain in weight was recorded for the male control group which received 30% of total calories as butter, but the experimental group at the lower fat level gained almost equally in weight (Fig. 4). The tall oil fatty acid glyceride margarine group on the 60% fat level did not thrive as well as in the first long-term experiment, but lagged far behind the other groups. There were highly significant differences ($P < 0.001$) between the groups receiving 60% of their total calories as butter or tall oil fatty acid glyceride margarine and between the tall oil fatty acid glyceride margarine groups on the lower

Table 17.

The effect of tall oil fatty acid glyceride margarine on weight gain of rats. Experiment II.

Type and amount of fat in the diet		Mean initial weight, g	Average cumulative weight gain, g — Week of experiment 1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	Deaths	Average food consumption, g (23 days)
Males														
Tall oil fatty acid glyceride margarine	30 cal %	42.6	21.1	52.0	85.4	113.1	139.1	166.8	185.4	208.6	216.3	227.6	0/9	343.1
Tall oil fatty acid glyceride margarine	60 »	34.3	6.2	17.4	28.9	46.7	67.0	93.4	114.5	130.2	144.1	155.7	1/10	220.2
Butter	30 »	50.6	25.4	61.4	86.6	114.4	141.1	168.5	187.7	209.0	216.7	232.4	0/10	337.4
Butter	60 »	44.0	12.6	34.8	59.5	81.6	113.4	138.4	163.4	185.7	203.7	219.5	0/10	269.7
Margarine	30 »	44.3	19.3	45.6	70.0	94.2	126.8	148.0	171.9	194.9	202.8	215.4	1/10	320.1
Margarine	60 »	37.8	13.5	35.2	52.0	73.1	103.7	127.0	151.7	176.8	196.0	201.6	0/10	262.4
Females														
Tall oil fatty acid glyceride margarine	30 cal %	44.7	17.6	45.4	65.9	85.1	103.2	117.3	126.8	135.4	141.7	144.6	0/20	279.2
Tall oil fatty acid glyceride margarine	60 »	31.6	5.7	17.6	28.0	46.3	62.3	77.6	90.9	101.5	105.3	111.9	0/20	204.5
Butter	30 »	48.1	18.5	45.8	65.5	79.7	97.6	109.4	116.1	127.6	132.0	132.2	0/20	269.4
Butter	60 »	39.7	11.2	31.0	49.7	61.4	81.3	97.7	108.1	118.8	125.9	129.1	0/16	227.3
Margarine	30 »	43.3	16.0	38.4	55.7	71.6	89.0	100.6	108.6	121.3	127.4	128.8	0/20	258.9
Margarine	60 »	35.0	10.6	32.3	53.0	68.6	86.1	102.3	118.6	128.9	135.3	137.3	0/7	227.0

and higher fat levels. A significant difference ($P < 0.01$) was found between the high-level butter and margarine groups, but not between the low-level butter and margarine groups, nor between the butter and tall oil fatty acid glyceride margarine groups.

The greatest growth in the female groups occurred, as in the former experiment, in the group receiving 30% of total calories as tall oil fatty acid glyceride margarine. The control groups receiving butter and margarine at both fat levels differed only slightly in their weight gains. The smallest weight gain occurred in the group receiving tall oil fatty acid glyceride margarine at the higher fat level. The difference between the control group receiving 60% of total calories as butter and the group receiving the same proportion of tall oil fatty acid glyceride margarine was highly significant ($P < 0.001$), but the difference was not significant at the lower fat level. A significant difference was found between the margarine group and the tall oil fatty acid glyceride margarine group on the lower fat level ($P < 0.01$) and a highly significant difference between the groups receiving tall oil fatty acid glyceride margarine at the lower and higher fat levels ($P < 0.001$).

In this experiment food consumptions were measured during three one-week periods. The results are presented in Table 17. In general, the food consumptions of the groups at the higher fat level were smaller than those of the groups at the lower fat level and the food consumption of the males was greater than that of the females. The only groups that consumed apparently less food than the control groups were those receiving 60% of total calories as tall oil fatty acid glyceride margarine.

The results indicate that the growth-promoting effect of the tall oil fatty acid glyceride margarine at the lower fat level was comparable with that of the control fats, but there was an apparent difference in growth between the control groups and the tall oil fatty acid glyceride margarine group on the higher fat level. This difference was more distinct in the experiment with the second generation than in the experiment with the first generation.

A third experiment (long-term experiment III) was conducted with the same kinds of diets, but with the offspring of the second generation. The experimental period was shorter, only five weeks. In addition to groups receiving butter, margarine or tall oil fatty acid glyceride margarine at levels of 30 and 60% of total calories, there were three control groups which received margarine made from refined (by redistillation in a vacuum) tall oil fatty acid glycerides with or without a supplement of B vitamins (biotin, pyridoxine, calcium pantothenate and folic acid) or only tall oil fatty acid glyceride margarine supplemented with the B vitamins. All these supplements were given at the higher fat level. The reasons for

Table 18.

The effect of tall oil fatty acid glyceride margarine on weight gain of rats. Experiment III.

Type and amount of fat in the diet		Mean initial weight, g	Average cumulative weight gain, g — Week of experiment 1.	2.	3.	4.	5.	Deaths	Average food consumption (7 days), g
Males									
Tall oil fatty acid glyceride margarine	30 cal %	38.9	10.4	29.3	52.3	79.2	103.9	0/10	60.7
Tall oil fatty acid glyceride margarine	60 »	37.5	1.1	11.0	15.4	24.2	33.8	0/10	35.6
Butter	30 »	44.1	29.4	70.2	104.9	146.6	176.5	0/10	109.2
Butter	60 »	44.6	23.0	55.5	82.8	121.5	162.9	0/10	81.6
Margarine	30 »	41.4	29.4	72.9	105.2	140.2	171.6	0/10	107.3
Margarine	60 »	49.5	18.4	51.2	75.9	109.4	144.2	0/10	88.8
Tall oil fatty acid glyceride margarine + vit. B	60 »	38.2	6.0	18.0	39.2	54.8	64.4	0/5	40.5
Refined tall oil fatty acid glyceride margarine	60 »	39.4	16.2	26.6	41.8	55.8	64.0	0/5	39.3
Refined tall oil fatty acid glyceride marg. + vit. B	60 »	39.8	19.0	30.8	52.8	72.2	82.4	0/5	46.3
Females									
Tall oil fatty acid glyceride margarine	30 cal %	39.5	12.7	33.2	51.0	68.3	79.4	0/10	62.6
Tall oil fatty acid glyceride margarine	60 »	31.5	3.8	11.0	14.6	21.2	28.0	0/10	28.0
Butter	30 »	39.5	25.8	53.9	74.0	96.9	108.6	0/10	87.6
Butter	60 »	45.6	19.0	46.4	62.2	88.2	108.2	0/10	71.3
Margarine	30 »	39.5	22.5	50.1	73.4	94.6	104.7	0/10	93.0
Margarine	60 »	46.5	18.3	40.2	64.1	85.7	104.6	0/10	82.3
Tall oil fatty acid glyceride margarine + vit. B	60 »	34.0	7.4	17.0	36.4	54.4	64.0	0/5	36.0
Refined tall oil fatty acid glyceride margarine	60 »	35.8	16.2	23.2	39.0	52.2	59.4	0/5	41.8
Refined tall oil fatty acid glycerido marg. + vit. B	60 »	34.0	16.4	28.2	45.2	61.0	70.8	0/5	44.9

refining the tall oil fatty acid glyceride margarine were the disturbances which were observed in the reproduction experiment with the second generation. It was suspected that the tall oil fatty acid glyceride margarine had become oxidized and that the oxidation products could partly be removed by redistilling the tall oil fatty acid glycerides. On the other hand, oxidized fats may destroy several of the B-group vitamins even in the animal organism.[58, 59] This gave cause to add the vitamins in question

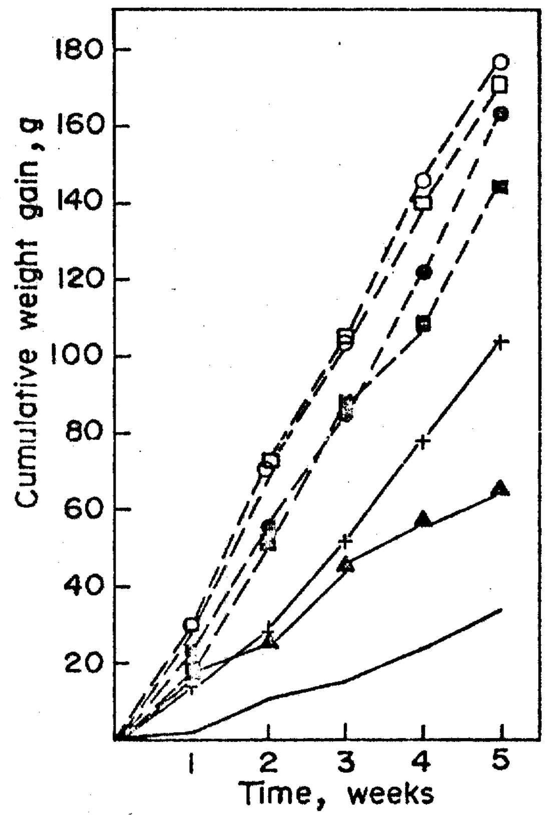

Fig. 5. Cumulative growth curves for male rats fed tall oil fatty acid glyceride margarine, butter and margarine. III generation.

—×——×——×—	30	cal %	tall oil fatty acid glyceride margarine
————————	60	»	» » » » » »
—▲——▲——▲—	60	»	refined tall oil fatty acid glyceride margarine
—○——○——○—	30	»	butter
—●——●——●—	60	»	»
—□——□——□—	30	»	margarine
—■——■——■—	60	»	»

to the diets of two control groups in approximately twice the amount which was supplied by the dried brewer's yeast in the basal diets.

The data of this experiment are presented in Table 18. The growth results indicate that the control groups at the higher and lower fat levels gained weight almost equally well, but that the experimental groups thrived much worse. The result was the same in both male and female groups. The diet containing both the refined tall oil fatty acid glyceride margarine and the vitamin B supplement had the best growth-promoting effect. The effects of refining the fat and adding the vitamin B supplement were

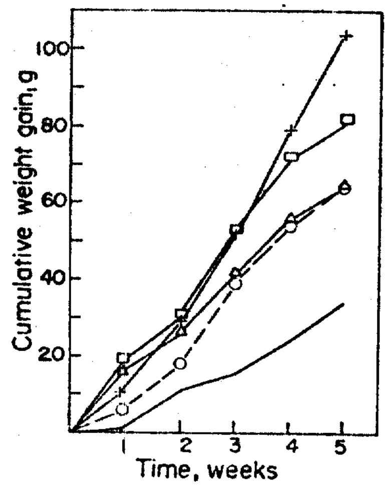

Fig. 6. Cumulative growth curves for male rats fed refined tall oil fatty acid glyceride margarine with or without vitamin B supplements. III generation.

—x—x—x 30 cal % tall oil fatty acid glyceride margarine
———— 60 » » » » » » »
—o- -o- -o— 60 » » » » » » » + B vitamins
—△—△—△ 60 » refined tall oil fatty acid glyceride margarine
—□—□—□ 60 » » » » » » » » + B vitamins

almost equal, although the vitamin B supplement was possibly more effective in the female group. The growth curves of the male groups are presented in Figs. 5 and 6.

There was a highly significant difference ($P < 0.001$) between the male group receiving 30% of total calories as butter and the male group receiving the same amount of tall oil fatty acid glyceride margarine. The difference was equally significant ($P < 0.001$) between the groups receiving butter and tall oil fatty acid glyceride margarine at the higher level. The difference between the groups receiving tall oil fatty acid glyceride margarine and refined tall oil fatty acid glyceride margarine at the 60% level was probably significant ($P < 0.05$), but the vitamin B supplement did not have a statistically significantly greater growth-promoting effect. The differences in the female groups were similar to those in the male groups, but some small differences were noted. The growth in the group receiving 30% of total calories as butter differed significantly ($P < 0.01$) from the growth in the group receiving the same amount of tall oil fatty acid glyceride margarine, but the difference was highly significant ($P < 0.001$) for the groups receiving these fats at the higher level. The group receiving the tall oil fatty acid glyceride margarine and the vitamin B supplement and the group receiving refined tall oil fatty acid glyceride margarine differed highly significantly ($P < 0.001$) from the experimental group receiving 60% of total calories as tall oil fatty acid glyceride margarine.

The food consumption was recorded during one week only, but, as can be seen from Table 18, the consumption corresponded well with the growth rates of the different groups. No deaths occurred in any of the groups during the experimental period.

More than half of the animals in the groups in the third long-term experiment were sent to the Department of Pathological Anatomy at the University of Helsinki for histopathological examinations (page 54).

b. Reproduction experiments.

Reproduction experiments give valuable information on the effects of a diet and its components. The breeding ability of the males and the fertility and reproductive performance of the females and their ability to suckle the young are dependent on, among other things, the dietary fat. During the strain of the gestation period, any deficiences in nutrition lead to disturbances in gestation or abnormalities in the offspring.

Several investigators[68, 69, 70, 71] have studied the effect of essential fatty acids on the reproduction of rats. The results indicate that linoleic and arachidonic acids are superior to linolenic acid as far as reproductive performance of the rat is concerned.

The reproduction experiments were carried out in connection with the long-term growth experiment with tall oil fatty acid glyceride margarine. In the first reproduction experiment 10 females and 5 males from each dietary group of the first generation were used. The offspring formed the second generation, which in turn was bred to obtain the third generation. The second reproduction experiment partly failed and had to be repeated (reproduction experiment III) with the same animals. From the litters of this third reproduction experiment, groups were formed to provide the third generation of the long-term experiment.

Experiment I. The diets were the same in this experiment as in the first long-term growth experiment. The data of the experiment are presented in Table 19. It will be observed that the rats of the tall oil fatty acid glyceride margarine groups reproduced even better than the rats of the control groups. All females on the lower tall oil fatty acid glyceride margarine level gave birth to normal litters and only two of the offspring died. Two females on the 60% level failed to become pregnant and the whole litter of one mother died. Half of the mothers in the control group receiving 60% of total calories as butter ate up their litters and only half of the females in the 60% margarine group had litters. Evidently two males in this group were sterile. A few cases of »ringtail» disease were observed in every group within ten days after the birth of the young. The animals did not seem to suffer from it noticeably, because they gained weight as well as their healthy littermates and none of them died from the disease. There were two possible explanations for the »ringtail» disease; a relatively low content of essential fatty acids in the diet and too low a relative humidity and temperature of the air and draught in the animal room during the winter-time.

Experiment II. As in the long-term experiment II, the diets were supplemented with 20 grams of soybean oil per kilogram to increase their content of essential fatty acids. Three additional groups were included in this experiment; one that received 30% and one 60% of total calories as margarine and the third 60% of total calories as tall oil fatty acid glyceride margarine without the soybean oil supplement.

The results are presented in Table 20. Marked differences are observed between the groups. The reproductive performance at the 30% fat level was almost normal and some of the litters were unusually large, but the number of dead young was apparently higher than in the first experiment. The difference between dietary groups was quite clear at the 60% fat level. Death of the offspring or »cannibalism» was quite common in all groups but most pronounced, almost 100%, in the groups that received 60% of total calories as tall oil fatty acid glyceride margarine with or without the soybean oil supplement.

Retardation and disturbances of growth were observed in a great number of the offspring. The hair was very thin and there were yellow spots, partic-

Table 19.

Reproduction experiment I. Number of litters and young in different groups and mortality percentages

Type and amount of fat in the diet	Number of females	Litters	Total number of young	Mean	Range	Number of dead young	Mortality %	Eaten
Tall oil fatty acid glyceride margarine 30 cal %	10	10	94	9.4	7–12	2	2.1	—
Tall oil fatty acid glyceride margarine 60 »	10	8	75	9.4	7–11	8	10.6	—
Butter 30 »	10	7	61	8.7	5–12	6	9.8	5
Butter 60 »	10	8	72	9.0	6–12	34	47.2	34
Margarine 30 »	10[1]	10	95	9.5	7–14	3	3.2	—
Margarine 60 »	10	5	49	9.8	7–12	2	4.1	—

[1] One female died.

ularly in the neck. An investigation made at the State Veterinary Laboratory indicated that the animals had an unspecific deficiency disease, characterized by the external signs mentioned above and by anemia and weakness of the skeletal muscles. Vitamin B or E deficiency was suspected to be the reason for the disease. As mentioned earlier, oxidized fat has a destructive effect on vitamins A, E and several of the B group vitamins.[59,60] Dehority *et al.*[58] obtained evidence of the inactivating effect of codliver oil on α-tocopherol in cattle.

The »ringtail» disease was not observed during this experiment, neither in the experimental groups nor in the control groups without added soybean oil in the diet. Because of this it was concluded that the cause of the »ringtail» in the former reproduction experiment was not a deficiency of essential fatty acids but rather draught, low temperatures and low relative humidities in the animal room.

Experiment III. Because no living third generation young were obtained from the 60% tall oil fatty acid glyceride margarine group in the preceding reproduction experiment, the experiment was repeated with the same animals. The animals in each group were divided into two subgroups *B* and *E*. The basal diets in the groups were type D or E. The diets of the *B*

Table 20.

Reproduction experiment II. Number of litters and young in different groups and mortality percentages.

Type and amount of fat in the diet	Number of females	Litters	Total number of young	Mean	Range	Number of dead young	Mortality %	Eaten
Tall oil fatty acid glyceride margarine 30 cal %	10	7	84	12.0	5–15	14	16.6	12
Tall oil fatty acid glyceride margarine 60 »	10[1]	7	59	8.4	7–12	57	96.6	17
Butter 30 »	10	10	104	10.4	9–13	14	13.5	4
Butter 60 »	10	10	100	10.0	6–12	33	33.0	19
Margarine 30 »	10	10	88	8.8	5–12	11	12.5	8
Margarine 60 »	7	7	65	9.3	8–12	24	36.9	12
Controls[2]								
Tall oil fatty acid glyceride margarine 60 »	10	8	74	9.3	8–11	74	100	26
Margarine 30 »	10	9	101	11.2	9–17	6	5.9	1
Margarine 60 »	10	10	101	10.1	6–14	12	11.9	8

[1] One female died.
[2] Diets without soybean oil supplement.

subgroups were supplemented with biotin, folic acid, calcium pantothenate and pyridoxine as in the third long-term experiment. The *E* subgroups received 0.15 gram of α-tocopherol dissolved in 2.5 grams of soybean oil per kilogram of diet. In addition, control groups were formed from the animals of the second long-term experiment. A group receiving 60% of the total calories as margarine was excluded because no females were available. The control groups received basal diets D or E containing 30 or 60% of total calories as butter, 30% of calories as margarine or 30 or 60% of calories as tall oil fatty acid glyceride margarine containing only the vitamin E supplement in the codliver oil.

The results are presented in Table 21. As the groups were relatively small, random factors may have affected the results. It will be observed that the vitamin B supplement had a favorable effect on the litter sizes and the survival of the young in almost all groups. An exception was the group that received 30% of total calories as tall oil fatty acid glyceride margarine and the vitamin B supplement, for two thirds of the young died or were eaten. The favorable effect of the vitamin E supplement was not

Table 21.

Reproduction experiment III. Number of litters and young in different groups and mortality percentages.

Type and amount of fat in the diet			Number of females	Litters	Total number of young	Mean	Range	Number of dead young	Mortality %	Eaten
Tall oil fatty acid glyceride margar.	30 cal %		5	5	50	10.0	8–12	10	20.0	6
» » »	30 »	+B[1]	5	5	64	12.8	9–16	40	62.5	28
» » »	30 »	+E[2]	5	5	47	9.4	3–13	25	53.1	21
» » »	60 »		5	4	33	8.3	6–14	33	100	11
» » »	60 »	+B	5	5	39	7.8	7– 9	11	28.2	6
» » »	60 »	+E	5	0	—	—	—	—	—	—
Butter	30 »		5	4	36	9.0	3–14	8	22.2	7
»	30 »	+B	5	5	62	12.4	9–14	20	32.3	10
»	30 »	+E	5[3]	4	45	11.3	8–12	10	22.2	4
»	60 »		5	3	21	7.0	2–10	8	38.1	7
»	60 »	+B	5	5	62	12.4	9–17	10	16.1	4
»	60 »	+E	5	3	21	7.0	2–12	11	52.4	3
Margarine	30 »		5	4	38	9.5	7–12	4	10.5	3
»	30 »	+B	5[3]	5	50	10.0	5–12	25	50.0	17
»	30 »	+E	5[4]	5	32	6.4	3–12	10	31.2	4
»	60 »	+B	5	5	41	8.2	4–11	4	9.7	3
»	60 »	+E	5	5	47	9.4	3–12	24	51.1	16

[1] B = vitamin B-supplement.
[2] E = vitamin E-supplement.
[3] One female died.
[4] Two females died.

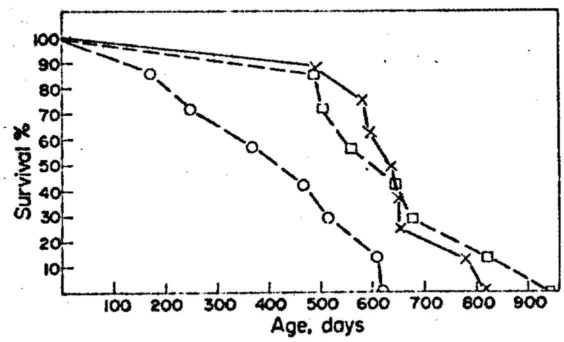

Fig. 7. Survival curves of male rats fed tall oil fatty acid glyceride margarine, butter and margarine.

—x—x—x—	30 cal %	tall oil fatty acid glyceride margarine
—o—o—o—	30 »	butter
—□—□—□—	30 »	margarine

as obvious as that of vitamin B. On the contrary, the litters were smaller and the mortality was greater in the *E* subgroups. In several of the *E* subgroups, half of the young died or were eaten by the mothers. The litter sizes and the number of surviving young in the control groups were almost normal with the exception of the 60% tall oil fatty acid glyceride margarine group in which all the young died. The group receiving tall oil fatty acid glyceride margarine at the 60% level and the vitamin E supplement was sterile.

No »ringtail» disease or other deficiency diseases were observed during this experiment.

c. *Longevity experiments*

The life spans of experimental animals have been shown to be longer when their food intake is restricted.[72] The same result is obtained when the intake of food is lowered by a poor appetite under *ad libitum* feeding conditions.[73]

The results of the longevity experiment with rats that received tall oil fatty acid glyceride margarine were not so clear, partly because of the small number of animals, but a certain tendency of the female rats receiving tall oil fatty acid glyceride margarine to have a longer life span than those receiving butter and margarine was observed.

Animals of the first long-term experiment which were left over from the reproduction experiment were kept for the longevity experiment. There were 7—9 animals per group at the 30% fat level. At the 60% fat

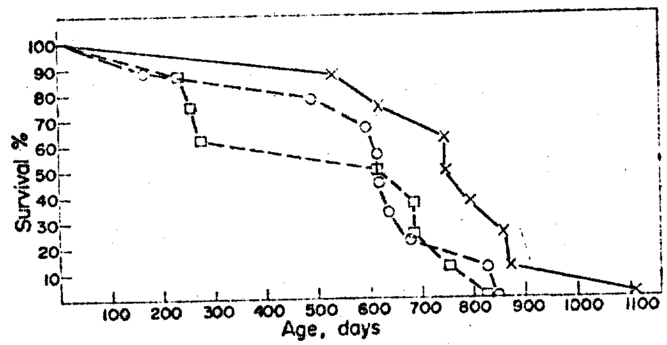

Fig. 8. Survival curves of female rats fed tall oil fatty acid glyceride margarine, butter and margarine.

—x—x—x— 30 cal % tall oil fatty acid glyceride margarine
—o—o—o— 30 » butter
—□—□—□— 30 » margarine

level only 3—4 animals per group were available, and hence the results for these are not presented. The diets were the same during the whole experimental period. The animals were weighed once a month and the deaths were recorded.

The survival curves are presented in Figures 7 and 8. It will be seen that the males in the low-level butter group died earlier than the males in the low-level margarine and tall oil fatty acid glyceride margarine groups. When all the butter-fed animals had died after 640 days, about 45% of the animals in the other groups were still alive. The curves of the ordinary margarine group and the butter group are linear but the curve of the tall oil fatty acid glyceride margarine group is not; it drops nearly vertically during the period from 600 to 650 days and crosses the ordinary margarine group curve. The survival curves of the females have different courses. The curves of the butter and margarine groups intersect, but they are almost similar in shape, although only 15% of the butter-fed animals had died when 37% of the margarine animals had died. The tall oil fatty acid glyceride margarine females had much longer life spans; the last animal died at the age of 1127 days.

The growth curves are presented in Figure 9. After an initial rapid increase, the average weights of the male groups reached a plateau where they remained for a period of about 12 months, after which the weight gains were more irregular owing to deaths, but mostly the trend was a declining one. The differences between the males of the tall oil fatty acid glyceride margarine group and the males of the control groups are not very clear. In the female groups the initial rise was not so abrupt, the plateau was reached earlier and the weight continued to increase slowly for about 15

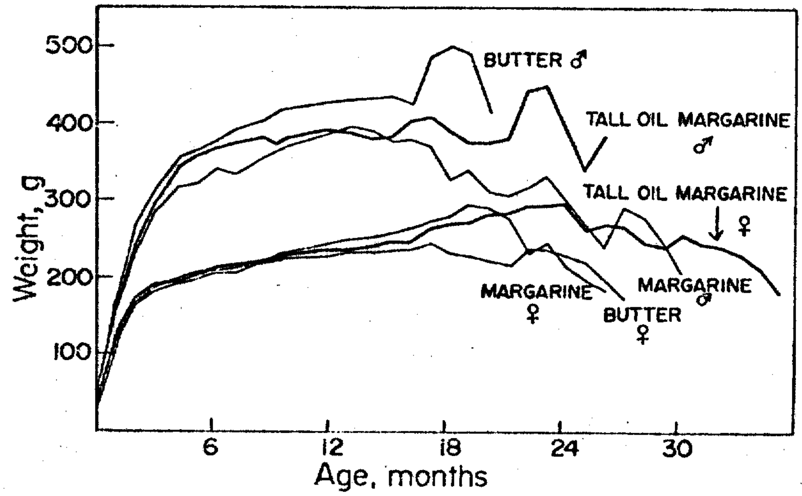

Fig. 9. Growth of rats fed tall oil fatty acid glyceride margarine, butter and margarine at a level of 30% of dietary calories in longevity experiment.

months. The weight then declined in the butter group, but the margarine and tall oil fatty acid glyceride margarine groups still gained weight, the former up to the 19th month and the latter up to the 24th month, when a decline began.

d. *Histopathological investigations*

In order to obtain information about any internal changes in the tissues of the rats that were fed tall oil fatty acid derivatives, animals of the third long-term experiment and animals that were given tall oil fatty acid glyceride margarine for the determination of fat absorption were examined at the Department of Pathological Anatomy, Division II, of the University of Helsinki under the supervision of Professor H. Teir.

The organs examined were the lungs, heart, thyroid gland, adrenal glands, liver, kidneys, stomach and part of the intestine (ileum and colon). The organs were first examined macroscopically and samples were fixed in 10% formaldehyde and cut into 5-μ sections, which were stained with Hemalaun-Eosin and van Gieson's stain.

Five male and five female rats from each test and control group in the third long-term experiment (p. 44), 90 animals in all, were examined for pathological changes. No greater differences were observed between the groups. The heart muscles were almost normal in the control groups and in the tall oil fatty acid glyceride margarine groups. In a few cases inflammatory cells were found, but they were of minor importance. The most marked changes were observed in the thyroid glands of animals of the margarine and tall oil fatty acid glyceride margarine groups, where a few cases of thyroiditis were diagnosed. Infiltration and fat necrosis had occurred in cells of some livers and the tubular cells of the kidneys of some animals of all groups were swollen.

Sixteen rats, both males and females, fed tall oil fatty acid glyceride margarine at the 30 and 60% levels in the fat absorbability experiment were also examined. One fourth of the animals examined exhibited slight degeneration of liver parenchyma and the tubular cells of half of the animals were swollen. Almost all groups included a few animals with peribronchal infiltrations in their lungs and a few cases of mild hyperplasia in the thyroid glands were observed. The adrenal glands were normal and no significant changes were observed in the hearts, stomachs or intestines of the animals.

The results indicate that on average the animals that were given tall oil fatty acids glyceride margarine had not suffered more serious internal lesions than the control animals in experiment III, but degenerative changes had occurred in the animals of the latter experiment, especially in the livers and kidneys.

e. The absorbabilities of tall oil fatty acids and their derivatives

The absorbabilities of tall oil fatty acid distillate, ethyl esters of distilled tall oil fatty acids and tall oil fatty acid glyceride margarine were determined. Three separate experiments were performed to determine the absorption of fats from diets containing tall oil fatty acid distillate at a level of 30% of total calories. The basal diet was type B. The methods of determining chromium in the feces and the average initial ages of the rats differed slightly in these experiments.

In the first experiment young male rats weighing about 40 grams at the beginning of the experiment were used. The control group received 30% of total calories as soybean oil. The feces of five animals from both dietary groups were collected separately and the absorbability of fats was determined by the chromium oxide method.[63]

The second experiment was conducted with older animals weighing about 250 grams. The control group was fed a diet containing soybean oil fatty acids and both this and the experimental group consisted of five male rats. The food ingested was weighed during 5 days and the feces of each rat were collected separately for fat content determination every day for 5 days after the day the diet was begun. The absorbability was determined simultaneously from the same fecal material by the ordinary chromium oxide method.

The chromium oxide was determined by atomic absorption spectrophotometry[64] in the third experiment. One group of ten male rats was fed tall oil fatty acid distillate and the control group comprising also ten male rats soybean oil fatty acids. The mean weight of the rats at the beginning of the experiment was 95 grams. The chromium content of the feces was determined on each of four days and twice on a sample collected during five subsequent days.

The absorbability of the ethyl esters of distilled tall oil fatty acids at the 30% level in the diet was determined using the chromium oxide method. The control group was fed soybean oil. The basal diet was type B. There were five male rats per group and the average initial weights of the rats were about 40 grams.

One experiment was carried out in which tall oil fatty acid glyceride margarine (prepared in the laboratory of the Department of Dairy Science, University of Helsinki) was given at the 30% and 60% fat levels. The basal diets were types B and C and butter was used as the control fat. Forty male and forty female rats were divided into eight groups of ten animals each (males and females in separate groups). The average initial weights of the animals of the groups were 41—44 grams. The absorbability of the dietary fats was determined using the chromium oxide method. The feces were collected separately from each rat during 8—20 days and duplicate determinations were made on each sample.

Table 22.

Absorbability of fat in male rats given tall oil fatty acid distillate, ethyl esters of distilled tall oil fatty acids, soybean oil and soybean fatty acids.[1]

Type and amount of fat in the diet		Age of rats	Method of determination	Number of samples	Mean absorbability percentage
Tall oil fatty acid distillate	30 cal %	young	chromium oxide[3]	5	96.7±0.32[2]
» » » » »	30 »	full-grown	» »	5	96.9±0.38
» » » » »	30 »	full-grown	food consumption	25	96.4±0.38
» » » » »	30 »	young	chromium oxide[4]	59	95.7±0.05
Ethyl esters of distilled tall oil fatty acids	30 »	young	chromium oxide[3]	5	93.1±0.96
Soybean oil fatty acids	30 »	full-grown	chromium oxide[3]	5	97.4±0.08
» » » »	30 »	full-grown	food consumption	25	97.6±0.49
» » » »	30 »	young	chromium oxide[4]	60	96.6±0.05
Soybean oil	30 »	young	chromium oxide[3]	5	98.1±0.32

[1] Uncorrected for endogenous fat excretion.
[2] Standard error of mean.
[3] Chromium determined by colorimetry.
[4] Chromium determined by atomic absorption spectrophotometry.

The data of the fat absorption experiments are presented in Tables 22 and 23. The fat absorption percentage given for each group is the mean of the determined values. The results indicate that the absorbabilities of tall oil fatty acids and tall oil fatty acid glyceride margarine are relatively good. The lowest absorption percentage, 93%, was obtained for the ethyl esters of distilled tall oil fatty acids. The average results for the tall oil fatty acid distillate are very similar for both young and adult rats, about 96–97% as determined by the ordinary chromium oxide method. The individual variations were, of course, larger, 1–2%. The results were almost the same, only 0.2–0.5% lower, when the food consumption method was used. The absorbability was found to be about 1% lower when the chromium oxide content of the feces was determined by atomic absorption spectrophotometry than when the ordinary chromium oxide method was employed. It is evident that the atomic absorption method is more sensitive and gives more accurate results.

As hydrogenated fats and saturated fats like butter are usually absorbed less effectively than oils, the same was expected to be true also for the tall oil fatty acid glyceride margarine, but the results indicate that the absorption of the latter was relatively high, 95–96%. The differences between the high and low fat groups were not significant, nor were the differences between the male and female groups significant. The absorbability of butter fat,

Table 23.

Absorbability of fat in young rats given tall oil fatty acid glyceride margarine and butter.[1]

Type and amount of fat in the diet		Sex of rats	Method of determination	Number of samples	Mean absorbability percentage
Tall oil fatty acid glyceride margarine	30 cal %	male	chromium oxide[3]	10	95.7±0.82[2]
Tall oil fatty acid glyceride margarine	30 »	female	» »	10	95.9±0.67
Tall oil fatty acid glyceride margarine	60 »	male	» »	10	95.2±0.75
Tall oil fatty acid glyceride margarine	60 »	female	» »	10	96.4±0.31
Butter	30 »	male	» »	10	94.3±0.62
»	30 »	female	» »	10	95.5±0.32
»	60 »	male	» »	10	92.9±1.15
»	60 »	female	» »	10	88.0±1.60

[1] Uncorrected for endogenous fat excretion.
[2] Standard error of mean.
[3] Chromium determined by colorimetry.

however, was lower than that of tall oil fatty acid glyceride margarine and it was 2–7% lower at the 60% fat level than at the 30% fat level. There was also a statistically significant difference ($P < 0.01$) between the male and female groups at the 60% butter level.

IV. EFFECT OF TALL OIL FATTY ACIDS ON TISSUE LIPIDS

A. Review of the literature on the effects of dietary fats on the fatty acid composition of tissue lipids

1. Adipose tissue

The predominant lipid class present in adipose tissue is the triglyceride class, which is usually considered the chief one in composition and fatty acid turnover studies. It is generally stated that dietary fatty acids alter the fatty acid compositions of depot fats and numerous investigations have dealt with the changes in fatty acid composition of adipose tissue in response to diet. The depot fats of aquatic animals have highly unsaturated fatty acid patterns, whereas the depot fats of land animals contain mainly palmitic and oleic acids. Ruminants represent a special class of land animals whose depot fats are modified by the action of rumen bacteria. In the following, mainly the adipose tissue fats of humans and rats are discussed.

Short-chain fatty acids ($< C_{12}$) are usually not stored as glycerides in the adipose tissues of rats to any greater extent,[74,75] although Gellhorn *et al.*[76] found nearly 5% decanoic acid in the adipose tissues of suckling rats. Knittle and Hirsch[77] investigated the effect of chain length on the incorporation of C_2 — C_{16} fatty acids into triglycerides of rat epididymal fat pads *in vitro*. Their results indicated that only small amounts of acids with very short chains are directly esterified to triglycerides, the major portion being elongated before esterification. Glycerides of fatty acids of intermediate chain length are deposited to a certain extent. Thus Longenecker[74] and Ostwald *et al.*[78] found 25–30% lauric acid among the fatty acids in the depot fats of rats fed a diet containing coconut oil.

The levels of long-chain saturated and polyenoic acids in adipose tissue lipids are readily altered by variations in dietary fat. When the diet is almost fat-free, the proportion of palmitic acid is nearly 30%, which represents fatty acid actually synthetized by the animals, but when the diet contains unsaturated oils, the dietary fat is reflected in the depot fat and the amount of palmitic acid is much lower.[79] The major component fatty acid of adipose tissue fats is usually oleic acid, which often amounts to over 40%. This acid seems to be a relatively constant component and the diet has less influence on its content than on the contents of other unsaturated fatty acids. This is due to the endogenous synthesis of oleic acid from saturated fatty acids, mainly stearic acid. Tove and Smith[80] observed that when the fat in the diet of mice contained 15% oleic acid, the level of this acid in the depot fat rose to 67%. This level of oleic acid was not exceeded although the content of oleate in the diet was more than doubled. When the level of oleic acid in the depot fat increased, palmitic acid was the only acid whose content decreased relative to the contents of other acids.

The most important group of fatty acids derived from the diet are the essential fatty acids, linoleic and linolenic acids. The linoleic acid content of adipose tissue increases rapidly when the dietary fat is rich in linoleate.[78,81,82] Tove and Smith[80] investigated the specific patterns of fatty acid replacement and observed that when the linoleic acid level in the depot fat increased to about 45%, palmitoleic acid was the only acid whose content decreased relative to the others, and a relative increase was noted in the amount of stearic acid. When the amount of linoleic acid in the depot fat rose above 45%, the contents of palmitic, palmitoleic and myristic acids decreased and the content of stearic acid increased. The investigations of Holman and coworkers on the dose-response of single dietary fatty acids revealed that the content of metabolites of linoleic acid rose with increasing dietary linoleate. Increasing amounts of dietary linoleic acid were able to suppress the content of C_{20} and C_{22} metabolites of linolenic acid, but relatively high concentrations were required to produce this effect.[83] Linolenic acid is not readily deposited in adipose tissue lipids, and only minor amounts of it have been reported to occur in human depot fats. Arachidonic acid is not stored in adipose tissue,[78] but other C_{20} acids have been detected, although in relatively small amounts.

Incorporation of odd-numbered and branched chain fatty acids into the adipose tissue lipids of humans has been observed by several investigators,[84–88] but usually only trace amounts of these acids have been found. Appreciable amounts of *trans* fatty acids have been detected in the lipids of human adipose tissue.[89,90,91] Oxygenated acids have been found by several investigators in adipose tissue lipids when diets containing epoxy acids and hydroxy acids have been fed to experimental animals.[92,93,94]

2. Blood lipids

Red blood cells contain considerable amounts of unesterified cholesterol. Monsen *et al.*[95] found levels that were three times higher than the level of total cholesterol in the plasma of rats. They observed that the amount of erythrocyte cholesterol was not affected by sex, dietary fat or level of cholesterol in the diet. Palmitic acid predominated over other saturated fatty acids in the triglyceride fraction of the erythrocytes, while palmitoleic and oleic acids were the main unsaturated components. The composition of the triglyceride fraction of erythrocytes remains apparently independent of the dietary fat. Phospholipids account for the greatest proportion of erythrocyte lipids. In contrast to their effect on the triglycerides, variations in the dietary fat cause appreciable changes in the fatty acid composition of the phospholipid fraction. Monsen *et al.*[95] observed increased amounts of linoleic and diminished amounts of oleic acid in the erythrocyte phospholipids of rats that had been given safflower oil in their diet. The quantity of arachidonic acid remained high and essentially constant regardless of the composition of the dietary fat. Hill *et al.*[96] observed similar changes in the phosphatidyl choline and serine fractions of human erythrocyte phospholipids when a diet rich in corn oil was consumed. No changes were observed in the fatty acids of the phosphatidyl ethanolamine and sphingomyelin fractions. The authors suggested that highly specific exchanges with plasma fatty acids lead to the alterations. Ways and Hanahan[97] found highly unsaturated fatty acids in the phosphatidyl ethanolamines of human erythrocytes.

Plasma lipids usually contain relatively high proportions of cholesterol esters and phospholipids and appreciable amounts of mono- and diglycerides, free fatty acids and free cholesterol, but the amount of triglycerides is relatively low. The plasma lipids are affected by the dietary fat to a considerable extent.[98,99] Oleic acid is the predominating fatty acid of plasma triglycerides according to Goodman and Shiratori,[100] but large amounts of palmitic and linoleic acids are also found. Plasma cholesterol esters contain large amounts of polyunsaturated fatty acids. Linoleic acid predominates in human plasma,[100,101] while arachidonic acid is the main fatty acid in the plasmas of other species such as the rat.[102] The phospholipid fraction is relatively constant in composition and is not affected by dietary fat although age, sex and growth rate are known to influence it.[78] The main component fatty acid in human plasma phospholipids is palmitic acid.[100,103,104] Monsen *et al.*[95] found that arachidonic acid is the main component in the plasma phospholipids of the rat. The amount of saturated acids is affected by sex differences; female rats maintained a higher proportion of stearic acid, whereas males had consistently more palmitic acid than did females. The different phospholipid classes show individual variations in fatty acid composition. According to Williams *et al.*,[105] the phosphatidyl choline and phosphatidyl serine fractions contain almost equal amounts of saturated and unsaturated fatty acids, whereas the fatty acids in choline plasmalogens and phosphatidyl ethanolamines are predominantly saturated.

3. Liver lipids

Triglycerides amount to nearly one third of the total liver lipids, but their content varies slightly depending on the type of dietary fat. The characteristics of the dietary fat are reflected in the fatty acid composition of the liver triglycerides; there are, however, marked differences between fatty acids which can be synthesized endogenously and those which are dependent on dietary sources. Okey *et al.*[106] detected nearly 9% lauric and myristic acids in liver triglycerides of rats fed a diet containing 10% coconut oil. However, the percentage of these acids in the liver never reached the percentages in adipose tissue triglycerides of the same animals, and evidence was found that the lower fatty acids are rapidly oxidized in the liver or transported to other tissues and are only partly stored as triglycerides in the liver. The finding of Sheig and Klatskin[107] that of labeled octanoic and palmitic acids, much less octanoic acid than palmitic acid was incorporated into hepatic lipids is in agreement with the previous results. Saturated acids remain at relatively constant levels regardless of the diet, but the percentages of oleic and linoleic acids vary greatly depending on the fatty acid composition of the dietary fat. Numerous investigations[106, 108, 109, 110, 111] concerning the incorporation of linoleic acid into liver and other tissue lipids after the intake of different amounts and types of dietary fats have revealed that dietary linoleate is reflected much earlier in liver neutral lipids than in liver total lipids or carcass lipids. Small amounts of arachidonic acid have been detected in liver triglycerides of animals fed safflower oil.[106] Observations of Holman and others[112–116] suggest that there is a competitive inhibition of the formation of higher metabolites in liver lipids by oleate, linoleate and linolenate.

The type of dietary carbohydrate has been found to influence the fatty acid composition of liver lipids. Thus, Casal and Holman[117] observed higher proportions of odd-chain fatty acids in liver lipids of rats fed a high-starch diet, while sucrose, glucose or maltose as a principal ingredient in the diet did not alter the normal fatty acid patterns of the liver lipids.

The cholesterol ester content of livers of rats on normal diets does not exceed 5% of the total liver lipids, but it is well known that excess cholesterol in the diet significantly increases the amount of esterified cholesterol. A cholesterol supplement led to the retention of a high proportion of linoleic acid in the livers of rats fed cottonseed oil, but decreased the proportion of linoleic acid retained in the livers of rats fed coconut oil low in linoleate. The latter rats evidently used higher proportions of oleic and palmitoleic acids in the esterification of cholesterol.[109] Liver cholesterol esters are similar to the liver triglycerides in fatty acid composition but contain much higher proportions of polyunsaturated fatty acids than the plasma cholesterol esters.[98,102] The percentage of myristic and lauric acids in the liver cholesterol esters never exceeded 5% regardless of diet,[106] but palmitic and stearic acids amounted to over half of the total fatty acids in liver cholesterol esters of rats fed a laboratory chow.[102] The linoleic acid content of liver cholesterol esters responds to changes in diet even more rapidly than its content in the plasma esters.[106] Williams *et al.*[105] observed a decrease in oleate in liver cholesterol esters of rats suffering from pyridoxine deficiency, but the arachidonic and linoleic acid contents were significantly higher in the cholesterol esters of the pyridoxine-deficient animals. These authors concluded that pyridoxine deficiency alters the metabolism of dietary and endogenous cholesterol.

The fatty acid composition of liver phospholipids is less sensitive to variations in diet than that of the other fractions. Phospholipids usually form over half of the total liver lipids,[111] but the proportion of total phospholipids tends to be lower in pyridoxine deficiency.[105] High proportions of stearic and arachidonic acids are characteristic of phospholipids. Okey *et al.*[106] found the proportion of arachidonic acid to be nearly 10% higher in the phospholipid fraction of the rat liver than in the plasma. Thirty-five to fifty per cent of the phospholipid fatty acids are saturated. A sex difference was observed by Okey *et al.*[106] in the chain lengths of the saturated fatty acids, stearic acid being present in high proportion in the phospholipids of female rats and palmitic acid in males.

The various phospholipid classes differ in fatty acid composition. The major phospholipid classes in rat liver are phosphatidyl cholines and phosphatidyl ethanolamines among both mitochondrial and microsomal phospholipids, which alone comprise more than 75% of the total phospholipids. About 45% of the fatty acids of the phosphatidyl cholines and ethanolamines in rat liver mitochondria and microsomes are the essential fatty acids, linoleic, C_{20} and C_{22} polyenoic acids, and particularly the percentages of arachidonic and docosahexaenoic acids are high in the phosphatidyl ethanolamine fraction of the microsomal phospholipids.[118] In animals on fat-free diets, the proportion of linoleic and arachidonic acids esterified in the β-position of mitochondrial phosphatidyl choline and ethanolamine decreases and that of palmitoleic and oleic increases significantly.[119] The predominating saturated acids esterified in the α-position are palmitic and stearic acids. The inositol glycerophosphatides of both mitochondrial and microsomal lipids contain over 70% stearic acid in the α-position and arachidonic acid is the main unsaturated acid.[119]

4. Fecal lipids

The composition of fecal lipids varies within relatively wide limits. According to Williams *et al.*,[123] 5 to 15% each of triglycerides, esterified cholesterol, unesterified cholesterol and phospholipids are found in samples of human fecal fat. The percentage of free fatty acids varies from 12 to 20. The fecal lipids include some unabsorbed dietary fat, but the major fraction is of endogenous origin or results from the synthetic action of intestinal microbes; the uncommon hydroxy acids and branched chain fatty acids are products of the metabolic action of microbes.[121] Investigations on the fatty acid composition of human fecal lipids have shown that palmitic and stearic acids each amount to over 30% of the total fatty acids.[120,121] Perkins *et al.*[93] investigated the effect of fresh corn oil fatty acids and hydroxy acids on the fatty acid compositions of carcass and fecal fats in the rat. They found that when fresh corn oil was given, the major component of fecal fatty acids was oleic acid, but when corn oil fatty acids were given, the content of linoleic acid was significantly higher than the content when corn oil was given, and even the amount of oleic acid was higher. Ricinoleic acid was rapidly eliminated in the fecal fats. The results of Uksila and Kurkela[123] on the fatty acid composition of the fecal fats of rats given diets which contained fresh or heated butter, lard or safflower oil revealed a correlation between the dietary and fecal fats. The linoleic acid content was lower in the groups that consumed heated fats than in the groups that consumed unheated fats.

B. Present investigations

1. Materials and Methods

a. Experimental animals and diets

The experimental animals whose tissue lipid fatty acid compositions were studied were those used in the experiments to determine the absorbability of tall oil fatty acids and soybean oil fatty acids. A separate group of rats given glyceryl esters of tall oil fatty acids was used for the study of fecal fats.

The fat in the diet amounted to 30% of the dietary calories. The type of diet was D, the detailed composition of which is given in Table 4.

b. Sampling and storage of tissues

The rats, which usually were fasted overnight, were rapidly anesthesized with ether ad. narcosin (Orion Oy). Each animal was placed on a small animal operation board and the abdomen opened. The blood was withdrawn from the *infernal vena* *cava* and centrifuged 15 min. at room temperature at about 2 500 rpm in a centrifuge tube containing heparin (Medica Oy) to prevent coagulation. The plasma was stored in a refrigerator until extracted a few days later. The liver was removed from the abdomen, blotted dry, weighed and immediately cooled and stored in an air-tight glass jar over Dry Ice until extracted. The adipose tissue samples were collected from around the kidneys or from the intestinal fat layers and stored in the same manner as the livers.

For the determination of fecal fatty acids, feces samples were collected in the experiments for the determination of absorbability and stored under nitrogen at −20°C until analyzed.

c. *Reagents and reference compounds*

Reagents	*Grade*	*Source*
Hydrochloric acid	37 per cent, d 1.19, guaranteed reagent	E. Merck AG
Potassium hydroxide	Guaranteed reagent	E. Merck AG
Silica gel G	For thin-layer chromatography	E. Merck AG
Sodium carbonate	Anhydrous, guaranteed reagent	E. Merck AG
Sodium sulphate	Anhydrous, guaranteed reagent	E. Merck AG
Sulphuric acid	95–97 per cent, d 1.84, guaranteed reagent	E. Merck AG
Acetic acid	99–100 per cent, d 1.05, guaranteed reagent	E. Merck AG
Acetic anhydride	Guaranteed reagent	E. Merck AG
Chloroform	Guaranteed reagent	E. Merck AG
Chromosorb W	Acid-washed, DMCS-treated 60/80 M.	Applied Science Lab., Inc.
Diazald	Research chemical	Aldrich Chem. Co. Inc.
Dichloro(R)fluorescein	Indicator	The British Drug Houses, Ltd.
Diethyl ether	Guaranteed reagent	E. Merck AG
Ethanol	Grade Aa	The State Alcohol Monopoly
Ethylene glycol succinate polyester	LAC 4-R-886	Wilkens Instr. & Res., Inc.
n-Hexane	Guaranteed reagent	E. Merck AG
Methanol	Puriss. pro analysi	Fluka AG
Petroleum ether	B.p. 40–60°C, AnalaR reagent	The British Drug Houses Ltd.
Pyridine	Guaranteed reagent	E. Merck AG

Reference compounds and mixtures	*Grade*	*Source*
Cholesterol	SCW	Nutritional Biochemicals Co.
Cholesterol oleate	Laboratory reagent	The British Drug Houses Ltd.
d, l-α-Lecithin, synthetic	Practical grade	Signa Chemical Co.
Oleic acid	Highly purified	Hormel Institute
Triolein	Highly purified	Hormel Institute
Methyl arachidonate	Highly purified	Hormel Institute
Methyl linolenate	Highly purified	Hormel Insitute
Methyl palmitoleate	Highly purified	Hormel Institute
Mixture of methyl esters of hexanoic, octanoic, nonanoic, decanoic and undecanoic acids	L-201	Applied Science Laboratories
Mixture of methyl esters of undecanoic, lauric, tridecanoic, myristic and pentadecanoic acids	L-202	Applied Science Laboratories
Mixture of methyl esters of pentadecanoic, palmitic, heptadecanoic, stearic and nonadecanoic acids	L-203	Applied Science Laboratories
Mixture of methyl esters of palmitic, stearic, oleic, linoleic and linolenic acids	GLC 1	Hormel Institute
Mixture of methyl esters of palmitic, nonadecanoic, heneicosanoic, eicosanoic and docosanoic acids	189-9	Sigma Chemical Co.

d. Analytical methods

Lipid extraction procedures

The adipose tissue was extracted by the method described by Söderhjelm and Söderhjelm[123] as modified in the Laboratory of Physiological Hygiene, University of Minnesota.[124] The tissue (2—4 g) was homogenized in 0.2 ml of concd. hydrochloric acid in a mortar. The homogenate was transferred with 95% ethanol to a Roehring tube and ethanol was added up to the 18-ml mark on the tube. Nitrogen was passed into the tube which was then inverted four times. The mortar was rinsed with 20 ml of ethyl ether, which was then added to the tube, which was flushed with nitrogen and then shaken for 1 min. Seven milliliters of distilled water and 25 ml of petroleum ether were added. Nitrogen was passed into the tube which was shaken again. The tube was allowed to stand until the upper layer was clear. This layer was filtered through a thin cotton layer into an erlenmeyer flask. The extraction was repeated twice in succession with 5 ml of ethanol, 15 ml of diethyl ether and 15 ml of petroleum ether. Nitrogen was used to remove air before each extraction. After the last extraction, so much water was added that the ether layer could be removed completely. The ethereal solution was evaporated under a stream of nitrogen on a water bath at 40°C. The lipid sample was stored under nitrogen at −20°C until analyzed.

The plasma lipids were extracted by the method of Krell and Hashim,[125] which is a modification of the Folch procedure.[126] The plasma (2—5 ml) obtained from each animal was injected with a glass syringe into a 50-ml volumetric flask containing 20 ml of a 2:1 (v/v) mixture of chloroform and methanol. The mixture was brought to the boil while being agitated, then cooled to room temperature and made up to volume with chloroform-methanol. The flask was thoroughly shaken by hand and the mixture filtered through a Whatman No. 3 filter paper that had been extracted fat-free with chloroform-methanol or petroleum ether. The fine protein precipitate that remained on the filter paper was discarded. The filtrate was collected in a 100-ml separatory funnel containing 15 ml of distilled water that had been equilibrated with chloroform-methanol. The filtrate and water were thoroughly shaken together and allowed to stand until two layers separated. The chloroform layer was transferred to an erlenmeyer flask and evaporated to dryness under nitrogen in a water bath at 50°C. The residue was stored in a desiccator in a refrigerator until analyzed.

The livers were dried in a vacuum oven at 60°C for 24 hours. They were then ground with quartz powder in a mortar and extracted by the Folch procedure[124] with 20 volumes of chloroform-methanol to one volume of liver tissue. The mixture was shaken in a 300-ml separatory funnel, washed with distilled water, equilibrated with chloroform-methanol and allowed to separate. The lipid extract was filtered through fat-free cotton wool into a Soxhlet flask and evaporated to dryness on a water bath at 50°C. The samples were stored under nitrogen at −20°C until analyzed.

Lipids were extracted from the feces samples by the method described by King.[65]

Thin-layer chromatography of tissue lipids

The lipid samples from different tissues were usually fractionated into phospholipids, cholesterol, mono- and diglycerides, free fatty acids, triglycerides and cholesterol esters by thin-layer chromatography.[125]

The glass plates were 200 × 200 mm and 4 mm thick. They were coated with a 0.25-mm-thick slurry of silica gel with a Desaga applicator (Desaga GmbH, Heidelberg). The slurry was prepared by shaking 35 g of silica gel and 70 ml of water in a stoppered erlenmeyer flask for 2—3 min. The coated plates were dried in air, activated at 100—110°C for one hour and stored in a desiccator until used.

The lipid extracts and reference compounds were applied onto the plates with a 5-μl Carlsberg micropipette. The reference compounds were d,l-α-lecithin, cholesterol, oleic acid, triolein and cholesterol oleate. The samples were pipetted on a line 1.5 cm from the lower edge of the plate; 5 samples each containing 1—10 μl of fat or reference compound were applied to each plate. Solid fats were first dissolved in a small volume of chloroform.

The solvent was a mixture composed of 25 volumes of diethyl ether, 2 volumes of glacial acetic acid and 73 volumes of n-hexane. The solvent was poured onto the bottom of the developing chamber to a depth of 0.5 cm. The front and back walls of the chamber were covered by filter paper to effect saturation of the air space.

Each run took 20—30 min. The plates were air-dried and immediately sprayed lightly with a 0.2% solution of 2'7'-dichlorofluorescein in ethanol. The phospholipid, triglyceride and cholesterol ester spots were located in UV light, marked with a sharp needle and quantitatively scraped into 50-ml erlenmeyer flasks for later analysis.

Gas liquid chromatography of tissue lipids

Saponification: The lipid samples fractionated on the thin layer plates were analyzed by gas liquid chromatography. The fractions, which were quantitatively scraped from the plates into 50-ml erlenmeyer flasks, were saponified with 10 ml of alcoholic potassium hydroxide (2 g of KOH in 50 ml of absolute ethanol) for 1 hour in a water bath at 82°C. Each flask was fitted with a reflux condenser and both were flushed with nitrogen before the saponification. After the solution had cooled, 30 ml of distilled water was added and the mixture was transferred to another flask so that the silica gel residues were left in the first flask. The liquid was extracted twice with 7 ml of petroleum ether to remove the unsaponifiable matter, which was discarded. The fatty acids were liberated with 3 ml of 10% hydrochloric acid, 10 ml of distilled water was added and the fatty acids were extracted with two 10-ml portions of petroleum ether. The extract was dried with anhydrous sodium sulphate.

Esterification: The filtered solution was evaporated under a stream of nitrogen and 4 ml of acidified methanol (1% w/v concd. sulphuric acid in absolute methanol) was added to the flask. The flask was attached to a reflux condenser and the flask and condenser were flushed with nitrogen before the liquid was refluxed two hours at 85°C. After it had cooled, the mixture was extracted with 10 ml of petroleum ether and the water was removed by suction. The petroleum ether layer was washed with 10 ml of water, which was removed by suction. The petroleum ether extract was dried overnight over a mixture of sodium sulphate and sodium carbonate (20:1) to remove water and possible acid residues. The solvent was evaporated under nitrogen and the methyl esters were transferred with a small volume of solvent to a semimicro centrifuge tube with a sharp point closed with a rubber or Teflon stopper and stored under nitrogen at -20°C in the dark.

Before chromatography, the solvent was removed with a stream of nitrogen.

Chromatography: Gas liquid chromatography was performed with a Perkin Elmer M 800 gas chromatograph. The columns used were either Golay columns (Perkin Elmer, Norwalk) 150' long and 0.01" in inside diameter coated with butanediol succinate or 2-meter-long stainless steel columns packed with Chromosorb containing ethylene glycol succinate. Before mixing with Chromosorb, the ethylene glycol succinate was dissolved in chloroform-ethanol (1:2 v/v) and passed through a column of Dowex 1 to remove any acid residues.[127] After evaporation of the solvent, the polyester was treated with acetic anhydride containing pyridine to esterify any free hydroxyl groups present. After evaporation of the solvent *in vacuo*, the polymer was suspended in ether and treated with diazomethane to esterify any free carboxyl groups remaining after the anion exchange. Chromosorb, 80 – 100 mesh, was impregnated with 15% of its weight of the dry polyester. The carrier gas was nitrogen and the flow rate approximately 30 ml/min. Samples 0.1 – 0.7 μl in volume were injected with 1-μl or 10-μl Hamilton syringes. The runs were temperature programmed from 160° to 190°C at a rate of 1.7°/min when Golay columns were used and from 140°C to 195°C at a rate of 3.3°/min when stainless steel columns were used. The peak areas were measured by triangulation.

Most of the fatty acids were characterized by direct comparison of the retention times of their methyl esters and those of the reference compounds. *Cis*-5,9,12-Octadecatrienoic acid was identified by means of its retention time read from a chromatogram of methyl esters of pine seed oil fatty acids.

2. Results

In the presentation of the fatty acid compositions of the various lipid fractions, the percentages of fatty acids containing less than 14 carbon atoms are summed. Distinct peaks were obtained with the columns used beginning from hexanoic acid, but the identification of the short chain acids was uncertain in some cases, obviously because some decomposition and oxidation of the methyl esters had occurred during the storage of the samples and peaks of new components possibly overlapped the peaks of esters of the short-chain acids in the chromatograms. Particular emphasis was laid on the determination of the C_{18} acids and especially *cis*-5,9,12-octadecatrienoic acid. Eicosanoic, eicosaenoic, eicosadienoic and eicosatrienoic acids are combined as C_{20} acids. Only eicosatetraenoic or arachidonic acid is presented separately because it is a major component acid of phospholipid fractions.

A few minor components could not be identified and their contents are given in the columns headed »other acids». They were in all probability unsaturated C_{14}, C_{15}, C_{16} and C_{17} acids. It is possible that some branched chain acids were present in the lipid samples.

Duplicate determinations were made whenever possible, but many of the samples were so small that it was possible to perform only one chromatographic run. Individual variations among the animals were considerable in some cases as can be seen from the standard errors of the means given in the tables.

a. Component fatty acids of adipose tissue lipids

The adipose tissue lipids were fractionated by thin-layer chromatography, but only the fatty acid compositions of the triglyceride fractions were determined. The results are presented in Table 24. The fatty acid compositions of lipids from both dietary groups resemble each other in many respects. The main component in both groups is oleic acid, which amounts to nearly half of the total acids. Some differences were, however, observed. The percentage of palmitic acid was slightly higher in the adipose tissue lipids of the animals that were fed soybean oil fatty acids than in those of the animals that received tall oil fatty acids. Differences are seen in the contents of palmitoleic, linoleic and stearic acids. The adipose tissue lipids of the group given soybean oil fatty acids contained 11.1% palmitoleic acid, 5.2% stearic acid and 5.8% linoleic acid; the corresponding values for the group given tall oil fatty acids were 2.1% palmitoleic acid, 2.4% stearic

Table 24.

Fatty acid compositions of adipose tissue triglyceride fractions from rats fed tall oil fatty acids and soybean oil fatty acids at a level of 30% of dietary calories.

	$<C_{14}$	C_{14}	C_{15}	C_{16}	$C_{16:1}$	C_{17}	C_{18}	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$[1]	$C_{18:3}$	C_{20}	$C_{20:4}$	Other acids
Tall oil fatty acids(6)[2]	1.1	1.4	0.3	18.9	2.1	0.3	2.4	47.4	20.7	1.0	0.6	1.5	0.0	2.2
S.E.[3]	±0.1	±0.1	±0.05	±1.6	±0.6	±0.07	±0.2	±2.0	±4.3	±0.3	±0.08	±0.5	±0.02	±0.05
Soybean oil fatty acids (4)	1.9	1.5	0.6	23.1	11.1	0.1	5.2	44.6	5.8	—	1.3	2.0	0.4	2.5
S.E.	±0.3	±0.2	±0.06	±4.4	±0.8	±0.06	±0.7	±4.1	±1.7	—	±0.3	±1.0	±0.3	±0.4

1 *cis*-5,9,12-Octadocatrienoic acid
2 Number of rats.
3 Standard error of mean.

acid and 20.7% linoleic acid. Only 1% *cis*-5,9,12-octadecatrienoic acid was stored in the adipose tissues of the animals that received the tall oil fatty acids. The other fatty acids, most of which were minor components, are of lesser importance. The adipose tissue fats of the rats appear to be relatively unsaturated, as nearly two thirds of the fatty acids in the adipose tissue fats of the soybean oil group and more than two thirds of the fatty acids in the adipose tissue lipids of the tall oil fatty acid group were unsaturated.

b. Component fatty acids of plasma lipids

The plasma lipids were fractionated into sterol ester and phospholipid fractions, which are the main fractions. Usually plasma contains small amounts of triglycerides, often less than 10%. Triglycerides were detected on the thin-layer chromatograms, but their amounts were too small for gas chromatographic analysis. Only three pooled samples of phospholipids and two pooled samples of sterol ester fractions from animals given tall oil fatty acids and one sample of each fraction from the animals that were given soybean fatty acids were analyzed.

The results are summarized in Table 25.

Cholesterol esters. The fatty acid composition of the cholesterol esters varies more readily than that of the phospholipids when different dietary fats are fed. The main component of the cholesterol ester fraction from the animals given soybean oil fatty acids was palmitic acid, while the percentage of stearic acid was relatively low (6.4%). Unsaturated C_{18} acids amounted to almost 40% of the total acids. The proportion of unsaturated C_{18} acids in the cholesterol esters of the group given tall oil fatty acids was over 50%, oleic acid alone representing 46.6%. Thus oleic acid was the main component in the plasma cholesterol ester fraction, while the percentage of oleic acid was much lower in this plasma fraction of the soybean oil group. Arachidonic acid was found only in the sterol esters of animals that were given tall oil fatty acids. The amount of *cis*-5,9,12-octadecatrienoic acid was somewhat higher in the cholesterol ester fraction (0.7%) than in the phospholipid fraction (0.3%).

Phospholipids. The phospholipids of both animal groups seem to be very similar in fatty acid composition. This is quite natural, as the phospholipid fraction is least affected by dietary changes. The main component in both groups was palmitic acid, which alone amounted to almost half of the total acids. The percentage of unsaturated C_{18} acids was less than 20 (18.5% in the group given soybean oil fatty acids and 18.1% in the tall oil fatty acid group). Only small differences were noted in the ratios

Table 25.

Fatty acid compositions of plasma lipid fractions from rats fed tall oil fatty acids and soybean oil fatty acids at a level of 30% of dietary calories.

	$<C_{14}$	C_{14}	C_{15}	C_{16}	$C_{16:1}$	C_{17}	C_{18}	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$[1]	$C_{18:3}$	C_{20}	$C_{20:4}$	Other acids
Cholesterol ester fraction														
Tall oil fatty acids (2)[2]	3.7	1.6	1.2	28.7	0.9	1.4	5.2	46.6	4.3	0.7	1.0	1.9	2.2	0.6
S.E.[3]	±0.6	±0.9	±0.05	±13.5	±0.3	±0.3	±1.6	±20.1	±3.2	±0.5	±0.01	±1.0	±2.2	±0.1
Soybean oil fatty acids (1)	0.8	1.6	0.4	49.5	1.5	0.1	6.4	14.0	23.7	—	1.5	0.2	0.0	0.3
S.E.	±0.3	±0.2	±0.03	± 1.0	±0.2	±0.1	±0.4	± 0.3	±1.9	—	±0.2	±0.2	—	±0.2
Phospholipid fraction														
Tall oil fatty acids (3)	2.1	0.6	0.7	43.5	0.5	1.2	27.0	10.2	7.2	0.3	0.4	0.6	4.8	0.9
S.E.	±0.6	±0.05	±0.09	± 4.1	±0.1	±0.3	±1.6	±2.4	±2.0	±0.2	±0.1	±0.4	±3.9	±0.3
Soybean oil fatty acids (1)	1.5	1.7	0.4	49.1	1.5	0.6	24.8	6.7	11.2	—	0.6	—	1.5	0.3
S.E.	±0.4	±0.1	±0.06	± 1.2	±0.1	±0.09	±0.5	±0.01	±0.7	—	±0.1	—	±0.5	±0.1

[1] *cis*-5,9,12-Octadecatrienoic acid.
[2] Number of samples.
[3] Standard error of mean.

of oleic acid to linoleic acid in the dietary groups. Small amounts (0.3%) of *cis*-5,9,12-octadecatrienoic acid were found in the phospholipid fractions of the animals that received tall oil fatty acids. A few percent arachidonic acid were detected, whereas only traces of arachidonic acid were found in the plasma lipids of the group given soybean oil fatty acids.

c. *Component fatty acids of liver lipids*

The liver lipids were fractionated into triglycerides, sterol esters and phospholipids. As the sterol ester samples of the tall oil fatty acid group were too small, the gas chromatograms did not give the true proportions of different fatty acids and thus only data for the triglyceride and phospholipid fractions are given. The chromatograms of the liver fatty acid esters were run on the capillary column.

The results are presented in Table 26.

Triglycerides. The major components of the liver triglycerides from animals fed soybean oil fatty acids were palmitic, oleic and linoleic acids, which each amounted to more than one fourth of the total acids. Small amounts of myristic, pentadecanoic, palmitoleic, stearic and C_{20} acids were present. The proportions of palmitic, oleic and linoleic acids in the liver triglycerides of the animals given tall oil fatty acids were a few percent lower. The content of stearic acid was only 3.3% in the soybean oil fatty acid group, but 13.8% in the group given tall oil fatty acids. The contents of other fatty acids were of the same order in both dietary groups except that the content of arachidonic acid was 3.8% in the group given tall oil fatty acids and 0.2% in the soybean oil fatty acid group. Over 2% *cis*-5,9,12-octadecatrienoic acid was found in the liver triglycerides of the animals given tall oil fatty acids.

Phospholipids. The fatty acid composition of the liver phospholipids is very similar in both dietary groups. The phospholipid fraction of the liver differs from that of plasma in the percentages of palmitic, stearic and arachidonic acids. The contents of oleic and linoleic acid are very similar to their contents in the plasma phospholipids. Palmitic acid forms more than one fourth of the total acids and stearic acid is the main component of both dietary groups, amounting to more than 40% of the total acids. The content of arachidonic acid was on average 8% in the liver phospholipids of both groups, but the variations in arachidonic acid content among the individual animals were great. Linolenic acid was detected only in traces or not at all, and 0.4% *cis*-5,9,12-octadecatrienoic acid was found in the phospholipids of the animals given tall oil fatty acids.

Table 26.

Fatty acid compositions of liver lipid fractions from rats fed tall oil fatty acids and soybean oil fatty acids at a level of 30% of dietary calories.

	$<C_{14}$	C_{14}	C_{15}	C_{16}	$C_{16:1}$	C_{17}	C_{18}	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$[1]	$C_{18:3}$	C_{20}	$C_{20:4}$	Other acids
Triglyceride fraction														
Tall oil fatty acids (8)[2]	2.1	0.8	0.6	23.4	1.4	0.5	13.8	19.3	27.4	2.1	0.3	1.0	3.8	3.5
S.E.[3]	±0.4	±0.08	±0.1	±0.8	±0.3	±0.05	±1.8	±1.6	±0.3	±0.07	±0.09	±0.1	±0.7	±0.6
Soybean oil fatty acids (8)	1.5	1.2	2.4	30.6	1.2	0.2	3.3	25.9	29.2	—	0.8	1.5	0.2	1.9
S.E.	±0.4	±0.1	±0.7	±2.4	±0.1	±0.02	±0.6	±1.0	±3.1	—	±0.2	±0.2	±0.09	±0.2
Phospholipid fraction														
Tall oil fatty acids (9)	2.3	0.2	0.5	26.5	0.3	1.2	40.8	9.7	8.2	0.4	0.0	0.1	8.0	1.8
S.E.	±0.5	±0.02	±0.06	±1.2	±0.04	±0.1	±1.1	±0.6	±0.8	±0.05	—	±0.03	±2.2	±0.2
Soybean oil fatty acids (6)	2.9	0.3	0.9	25.4	0.3	0.7	42.6	6.5	10.9	—	0.2	0.8	7.6	0.9
S.E.	±1.0	±0.04	±0.4	±1.7	±0.04	±0.1	±3.3	±0.2	±1.7	—	±0.07	±0.1	±4.5	±0.1

1 *cis*-5,9,12-Octadecatrienoic acid.
2 Number of rats.
3 Standard error of mean.

Table 27.

Fatty acid composition of fecal lipids from rats fed glyceryl esters of tall oil fatty acids at a level of 30% of dietary calories.

	$<C_{14}$	C_{14}	C_{15}	C_{16}	$C_{16:1}$	C_{17}	C_{18}	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$[1]	$C_{18:3}$	C_{21}	$C_{20:4}$	Other acids
Mean percentage	1.1	0.6	0.8	4.9	1.3	0.7	2.8	25.3	19.7	16.2	8.3	2.6	1.0	14.7
S.E.[2]	±0.2	±0.1	±0.08	±0.3	±0.1	±0.1	±0.1	±1.0	±0.8	±0.5	±0.3	±0.4	±0.4	±0.8

[1] *cis*-5,9,12-Octadecatrienoic acid

[2] Standard error of mean.

d. Component fatty acids of fecal lipids

The fecal lipids of a group of adult male rats given 30% of total calories as glyceryl esters of tall oil fatty acids were analyzed for fatty acid composition. Random fecal samples were collected from 10 rats during a three-week period and stored under nitrogen until analyzed. The total fatty acid compositions were determined without fractionating the lipids into classes. The results are presented in Table 27.

The contents of saturated acids were relatively low, about 10%. The predominant acids were oleic and linoleic acids; both were found in an amount of 20% or more. The most striking feature in the composition of the fecal fatty acids was the high content of *cis*-5,9,12-octadecatrienoic acid, about 16% of the total fatty acids. This is markedly more than was detected in any of the examined organ lipids from the rats fed tall oil fatty acids. All other component fatty acids were present in quantities less than 10%. Several unidentified acids, which were probably formed by the action of intestinal flora, were included in the group of »other fatty acids».

V. DISCUSSION

The possibility of using refined tall oil fatty acid derivatives as edible oils and fats has been mentioned earlier by several investigators.[32,33,34,39,40] Antila and coworkers investigated the effects of ethyl esters of tall oil fatty acids mixed with fodder for milch cows and poultry. They found that the iodine value of milk fat increased when tall oil fatty acid ethyl esters were given, whereas the fat content and iodine value of egg yolk remained unchanged. Seppänen *et al.*[128] investigated the effect of tall oil fatty acid glycerides on the growth of rats and found that at least small amounts of hydrogenated glycerides could be used in the diets of experimental animals without endangering their health.

In the present study the effects of tall oil fatty acids and their ethyl and glyceryl esters on the growth rate, reproduction and longevity of rats were investigated. Different derivatives of tall oil fatty acids were given in the diets usually at a level of either 30% or 60% of dietary calories in both short-term and long-term experiments. Growth was almost normal in several experiments with the lower fat level, but the growth of many of the experimental groups on the higher fat level was retarded or the weights dropped below the initial level. This was very marked especially when diets containing less refined tall oil derivatives were given. On the other hand, the animals in the control groups on diets which contained 60% of total calories as soybean oil or butter grew almost as well as the control groups given diets containing these fats at a level of 30% of total calories.

The amount of fat normally used in commercial rat diets is relatively low, only 4—5 wt%, but much higher percentages have been used in experimental diets. As shown by the results of the first growth experiment where tall oil fatty acids and soybean oil were compared, the optimum fat level for the rat seems to be nearer 30 cal% (13 wt%) than 15 cal% (6 wt%). The rat is able to digest comparatively large amounts of fat, as found in the experiments where the tall oil fatty acid glyceride margarine was given at a level of 60% of the dietary calories and 96% was absorbed.

The degree of refinement of the tall oil fatty acid esters had a marked influence on the growth rate of the animals. In general, the more refined products resulted in more rapid growth. All the animals that were given the higher level of the tall oil fatty acids died within a few days. On the other hand, tall oil fatty acid glycerides refined by the usual methods employed in the manufacture of edible oils supported growth even at the higher level, and growth at the lower level was almost comparable with that of the control groups. Hydrogenation of the tall oil fatty acid glycerides seemed to improve the growth-promoting effect, and this effect was further increased when the hydrogenated tall oil fatty acid glycerides were interesterified with hydrogenated soybean oil to form a margarine-type fat.

Elevated temperatures used in sulphate pulping and the distillation of tall oil certainly cause polymerization of the fatty acids in tall oil with the simultaneous formation of toxic substances. Data of Crampton *et al.*,[129] Johnson *et al.*,[130] and Kaunitz [131] show that even small amounts of thermally polymerized fats given to rats seem to depress the growth of the animals and cause diarrhea and skin disorders. When higher concentrations of the polymeric fractions of fats were fed to animals, these all died within a few days.[132] Especially cyclic monomers which are easily formed during the polymerization of linolenate appear to be more toxic than the polymers.[133] The results of the present experiments, in which it was found that tall oil fatty acid distillate and ethyl esters of distilled tall oil fatty acids strongly depressed the growth of rats and caused other symptoms similar to those mentioned above, suggest that these less refined tall oil products contained polymerized fatty acids.

The formation of cyclic monomers is possibly the explanation for the toxicity of *cis*-5,9,12-octadecatrienoic acid, an isomer of linolenic acid, and the lethal effect of the fraction 4 that did not form urea adducts when these were given to rats. In contrast, pine seed oil, which was not thermally degraded, promoted normal growth although it contained more than 20% of the same acid.

The possible presence in tall oil fatty acid products of a chlorinated hydrocarbon as a toxic factor of the »chick edema factor» type was also suspected, since it has been found in distilled tristearin [134] and oleic acid[135] and has been stated to cause edema symptoms also in rats.[136] Firestone *et al.*[137] succeeded in isolating the »chick edema factor» from tall oil. Final conclusions about the role of the »chick edema factor» in the retardation of growth cannot, however, be drawn on the basis of the present investigations.

Food consumption, which was also recorded in most of the growth experiments, was usually in close agreement with the growth data. As the food was given *ad libitum*, it may be assumed that the palatability, among other things, affected the food consumption. Particularly the rats that were given ethyl and glyceryl esters of the tall oil fatty acids consumed ery little food, and this was reflected in the growth rates of the animals. The food consumption might be a better basis than the growth rate for the evaluation of the different tall oil derivatives as food components, but usually the growth rate gives a clearer picture of the course of a feeding experiment.

The reproduction experiments which were carried out with animals of the first generation that were fed tall oil fatty acid glyceride margarine resulted in normal litters. The offspring of the animals of the second generation suffered from serious disturbances in the skin and skeletal muscles and died or were eaten up by the mothers within two weeks. The symptoms resembled those of muscular dystrophy. The cause of these disturbances was concluded to be connected with the oxidation of the tall oil fatty acid glyceride margarine and the partial decomposition of certain B vitamins by the oxidation products. This conclusion received support from the favorable effect of the addition of biotin, folic acid, calcium pantothenate and pyridoxine to the diet. An antagonistic effect of codliver oil on vitamin E was not demonstrated, as elevated vitamin E levels did not improve reproduction in the experimental and control animals.

The female rats that were fed on diets containing tall oil fatty acid glyceride margarine had longer life spans than the control animals, and the male rats were nearly as long-lived as the animals fed on diets containing ordinary margarine. Longevity is often connected with restricted feeding and low body weight, and this seemed to be true in the case of the male rats that received tall oil fatty acid glyceride margarine. However, the female rats gained as much weight on average and had longer life spans than the control animals.

As to the use of tall oil fatty acids for dietary purposes, it seems obvious that the main problem to be solved is the efficiency of the refining process. Refining and hydrogenation seem to improve the product in general, and the hydrogenated product may be more suitable as a raw material for food fat for human consumption. For the present, the manufacturing process cannot be changed very much, but if the impurities can be removed, tall oil fatty acids no doubt will be a valuable source of edible fats.

The composition of dietary fats is reflected in the composition of tissue lipids, but the actual replacement depends on a number of factors and varies considerably from one tissue to another and from one lipid class to another.

The content of palmitic acid in the adipose tissues of rats fed on a low fat or an almost fat-free diet is about 30%, but when more unsaturated oils are fed, the percentage of palmitic acid is often substantially lower.[138] This was also observed in the present investigation, for 23.1% palmitic acid was found in the adipose tissue lipids when soybean oil fatty acids were given, but somewhat less, 19%, when tall oil fatty acids were given. The fatty acid that predominated in the depot fats of both dietary groups was oleic acid; its content was 45% in the soybean oil fatty acid group and 47% in the tall oil fatty acid group. This is in agreement with the results of Beare and Kates,[110] but not with those of Tove and Smith,[80] who found that when the diet of mice contained 15% oleic acid, the level of oleic acid in the depot fat was 67%. The soybean oil fatty acid mixture contained about 20% and the tall oil fatty acid distillate 35% oleic acid, which is more than twice the level in the fats given by Tove and Smith. The content of linoleic acid in the adipose tissues of the group given tall oil fatty acids was almost four times higher (20.7%) than the content in the adipose tissues of the soybean oil fatty acid group (5.2%), which is not in agreement with the higher content of linoleic acid in soybean oil. On the other hand, the amount of palmitoleic acid was about 11% in the adipose tissues of the soybean oil fatty acid group and only about 2% in the adipose tissues of the tall oil fatty acid group. One percent *cis*-5,9,12-octadecatrienoic acid was detected in the adipose tissues of animals fed tall oil fatty acids. The other components were usually present only in minor quantities.

The fatty acid compositions of plasma and liver triglycerides resemble the fatty acid composition of the adipose tissue triglycerides, but the cholesterol esters and phospholipids differ significantly in fatty acid composition.[98,102,139,140] The plasma triglycerides, which are present only in small amounts in the blood of fasting animals, were not analyzed in the present study, but the liver triglycerides were analyzed.

The results for the liver triglycerides agree well with the fatty acid composition of liver triglycerides of rats given a cottonseed oil diet reported by Okey *et al.*[109] Only the content of linoleic acid was considerably higher in the livers of the latter group (about 40%) than in the livers of the rats fed tall oil fatty acids and soybean oil fatty acids (25—30%). The content of linoleic acid is of the same order in cottonseed oil and soybean oil and hence the dietary sources of these acids were almost equivalent, although some deterioration of the linoleic acid may have occurred during the storage of the esterified fatty acid samples. The major difference between the two dietary groups was found in the percentage of stearic acid, which was almost five times greater in the liver lipids of the tall oil fatty acid group than in the liver lipids of the group given soybean oil fatty acids. Slightly more *cis*-5,9,12-octadecatrienoic acid was present in the liver triglycerides than in the adipose tissue triglycerides of the former group.

The cholesterol esters of liver lipids usually resemble the liver triglycerides in fatty acid composition,[102,109] whereas plasma cholesterol esters contain higher amounts of polyunsaturated fatty acids. Swell *et al.*[102] and Monsen *et al.*[95] reported very high percentages of arachidonic acid in the cholesterol ester fraction of rat plasma when ordinary laboratory chow or a diet containing safflower oil was given to the animals. This is not in agreement with the results of the present study, for palmitic and oleic were found to be the main component fatty acids. Only minute amounts of arachidonic acid were detected in the plasmas of the two groups. This was possibly due to the oxidation of the arachidonic acid during the long time the esters were stored before they were analyzed in the gas chromatograph although they were stored at $-20°C$ under nitrogen. The average content of *cis*-5,9,12-octadecatrienoic acid in the cholesterol ester fraction of the plasma was 0.7%.

The phospholipids of plasma and liver appear to have low contents of monoethenoid acids, mainly oleic acid, and high contents of stearic and arachidonic acids.[79,99] The liver phospholipids had much higher contents of stearic acid than the plasma phospholipids, whereas the percentage of palmitic acid was much higher in the plasma phospholipids than in liver phospholipids. The content of arachidonic acid (8%) in liver phospholipids differs clearly from the percentages (35—40%) given by Okey *et al.*,[106,109] and the difference is even greater in the case of plasma phospholipids. The reason is most probably the oxidation of the arachidonic acid in the samples stored before the gas chromatographic runs, but may also be that the liver lipids were analyzed using a capillary column and the plasma lipids in a packed column which may have affected the results. The fatty acid compositions of the plasma and liver phospholipids in the groups given tall oil fatty acids and soybean oil fatty acids differed only slightly. The content of *cis*-5,9,12-octadecetrienoic acid was less than 0.5% in both the plasma and liver phospholipids, and thus nearly equal to the content of linolenic acid in the two phospholipid fractions.

Two peaks appeared where the peak of oleic acid was expected in the chromatograms of methyl esters of the fatty acids of the phospholipid fractions from rats fed soybean oil fatty acids. These may have been due to the separation of the *cis* and *trans* forms of octadecaenoic acid, as these isomers are separated when a »BDS» capillary column is used for separation.[91, 141] This was, however, the only fraction in which the isomeric forms were distinctly separated, although all the liver lipid fractions were separated on the same column.

cis-5,9,12-Octadecatrienoic acid, which was detected in all tissue lipid fractions from the animals given tall oil fatty acids, accumulated in adipose tissue, but only to a relatively small extent. It was present in an amount of 2% in the triglyceride fraction of the liver lipids. The positions of the double bonds in the acid ($C_{18:3}$ n-6,9,13) indicate that it resembles the members of the linoleic acid family ($C_{18:2}$ n-6,9) with the first double bond between the 6th and 7th carbon atoms from the terminal methyl group, but because of the abnormal location of the third double bond from the methyl group, it seems hardly possible that it could be metabolized according to the linoleic acid pathway. It is more likely that *cis*-5,9,12-octadecatrienoic acid is metabolized further along the pathway of fatty acids with even carbon chains although this was not confirmed in the present investigation. Only minute amounts of this acid were detected in the blood lipids and liver phospholipids, but the large amount (16%) detected in the fecal fat indicates that a considerable amount was not absorbed within the organism.

The present results indicate that the dietary fats have a marked influence on the depot fats and on the triglyceride fraction of liver lipids. Feeding of fatty acids instead of glycerides may have affected the fatty acid composition of the tissue lipids, but the changes were presumably relatively small, as indicated by the experiments of Perkins *et al.*[93, 94] in which corn oil and corn oil fatty acids were given to rats and only minor differences were observed in the fatty acid compositions of the carcass fats.

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Pharmaceutical-Grade Sterols from Tall Oil[1]

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During recent years there has been an increasing emphasis on medical research in the field of arteriosclerosis, popularly known as hardening of the arteries, and related disorders. Atherosclerosis, one form of arteriosclerosis, is characterized by the deposition of fatty matter on the inner walls of arteries. Atherosclerosis is the major factor in coronary artery disease and cerebrovascular accidents, popularly known as strokes. Although many theories have been advanced, the mechanism of the deposition of this fatty matter is still conjecture.

It is generally agreed among the medical profession that cholesterol, the predominant sterol found in animals, plays an important part since cholesterol is a major component of atherosclerotic deposits. Evidences have been produced which show that persons with atherosclerosis and related diseases may have a higher blood serum cholesterol content, known as hypercholesteremia, than those persons apparently free of artery disease (1). A reduction in cholesterol serum levels for hypercholesteremia patients appears desirable. However cholesterol is a very necessary ingredient for proper bodily functions. The body receives its cholesterol from two sources. Cholesterol is synthesized by the body and is absorbed from food sources found in a well-balanced diet. A lowering of serum cholesterol level sometimes can be attained by strict diet. Dieting is undesirable for the required type of diet is monotonous and unpalatable by American standards.

An oral intake of sitosterols, sterols found in vegetable and fruit sources, can be effective in reducing the level of serum cholesterol in patients with high cholesterol levels (2). The mechanism that causes this reduction is not known. One theory uses as an explanation that sitosterols interfere with absorption of cholesterol, possibly by the formation in the intestinal tract of a mixed crystal of sitosterol and cholesterol whose solubility is considerably less than that of cholesterol alone (3, 4). Other theories of the mechanism have been advanced. Figure 1 shows the chemical similarity of cholesterol, betasitosterol, and dihydrositosterol.

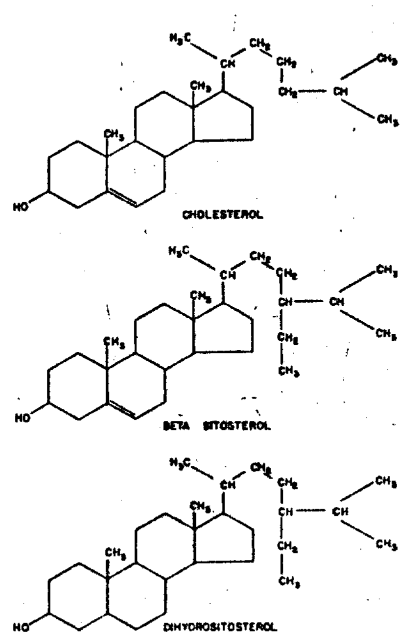

FIG. 1.

A commercial preparation of sitosterols for oral intake has been introduced. This preparation is a 20% suspension of betasitosterol and dihydrositosterol. The introduction of this therapeutic agent to lower serum cholesterol levels became possible when commercial quantities of suitable sitosterols made from tall oil were offered by Swift and Company. Betasitosterol, which seems to be the desired sterol for this application, is found widely distributed in vegetable sources. Particularly good sources for betasitosterol are tall oil and cottonseed oil. The composition of the sterols in these oils is 80% to 85% betasitosterol, 15% to 20% dihydrositosterol, and minor amounts of other sterols.

[1] This paper won first place in the 1958–59 Tall Oil Award of the Tall Oil Division of the Pulp Chemicals Association.

Sterols are concentrated in the foots in the alkali refining of vegetable oils. They are further concentrated upon distillation of acidified foots. Still bottoms from the distillation of cottonseed foots contain 7% to 10% total sterols. The sterol content varies quite widely depending upon the source, the refining, and distillation of the foots.

TALL OIL PITCH, resulting from the distillation of crude tall oil, offers the richest source of beta-sitosterols. Production of crude tall oil had reached 550,000,000 lbs. in 1955 (5) and production has increased continually. Of the crude tall oil produced and distilled in the United States, 15% to 25% results in pitch. Tall oil pitch is an inexpensive commodity and, in general, is in oversupply.

It may be conservatively estimated that the potential demand for sterols as a therapeutic agent for atherosclerosis could reach volumes requiring well over 50,000,000 lbs. of tall oil pitch per year. This assumes widespread acceptance by the medical profession and subsequent consumption by several million Americans who could derive some benefit from its use.

TABLE I

Typical Tall Oil Pitch Analysis

Color	Black
Acid number	65
Saponification number	110
Iodine number	140
Rosin acids	30%
Fatty acids	35%
Unsaponifiable matter	32%
Ash	0.05%
Softening point	90°F.

A typical analysis of tall oil pitch is shown in Table I. The composition of tall oil will vary considerably among producers. Since the primary concern was for the sterol content of tall oil pitch from different suppliers, samples from a number of suppliers were analyzed for unsaponifiable matter and total sterol content with results as shown in Table II. Satisfactory sterols could be made from each of the several sources listed, but yields drop with lower initial sterol content.

TABLE II

Unsaponifiable Matter and Total Sterol Content of Tall Oil Pitches

Source	Unsaponifiable matter	Total sterols
	%	%
1	32.0	16.0
2	26.0	12.0
[illegible]	27.3	10.5
[illegible]	31.0	6.8

The literature reveals a number of methods for the recovery of sterols (6, 7, 8, 9). Essentially these processes resort to saponification of the sterol source and extraction of the unsaponifiable matter with a suitable solvent. The unsaponifiable matter is recovered and dissolved in another solvent and crystallized therefrom. Usually one or more recrystallizations are necessary to produce a relatively pure product. Hickman (10) resorted to molecular distillation for purification. Generally the processes disclosed were found to be inadequate. Sterols produced by the foregoing methods do not meet the specifications, Table III, particularly on color and taste for pharmaceutical-grade sterols.

TABLE III

Pharmaceutical-Grade Tall Oil Sterol Specifications

Chloroform-insoluble	0.1% max.
Dirt	10 ppm. max.
Color (opt. den. @ 400 mμ)	0.100 max.
Melting point	134 to 140°C.
Specific rotation	—25 to —38°C.
Moisture and volatile	2% max.
Ash	1% max.
Heavy metals	20 ppm. max.
Sodium	0.1% max.
Methanol	0.0 ppm.
Total sterols[a]	95% min.
Unsaturated sterols	85% min.
Taste	Bland
Particle size	1-in. diam. max.

[a] By digitonin assay.

After an intensive laboratory and pilot-plant investigation, processing facilities to produce commercial quantities of pharmaceutically-pure sterols were installed at Swift and Company's Technical Products Plant at Hammond, Ind. Figure 2 shows a flow diagram of this process (11).

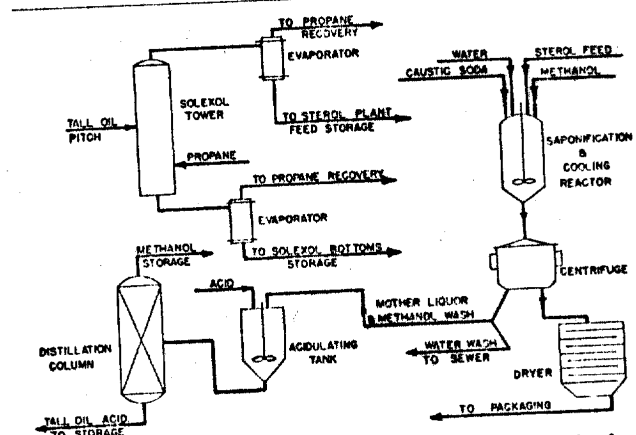

FIG. 2. Flow diagram. Pharmaceutical grade sterols from tall oil pitch.

The first step in the process, that of fractionation or extraction using liquid propane (Solexol process) (12), results in a light-colored (11C FAC) liquid overhead fraction. This overhead fraction contains 22% to 25% total sterols plus higher alcohols, fatty and rosin acids, and esters of fatty acids. The bottoms fraction has a ring and ball-softening point at 115°F. and consists principally of the oxidized polymerized components of the tall oil pitch. It has been found to have advantages for use in the same general areas that tall oil pitch has found application.

To accomplish the above fractionation the tall oil pitch is fed to the Solexol tower at a ratio of 20:1, solvent to pitch, at a tower temperature of 180°F. and a pressure of 680 psig. The fractionation results in approximately 50% each of overhead and bottoms. The composition of the tall oil feed stock may necessitate changes in operating conditions.

The removal of the oxidized and polymerized components from tall oil pitch is necessary for the production of pharmaceutically-pure sterols. The omission of the propane fractionation results in a product containing unknown ingredients which impart undesirable color and taste to the final sterol product. In addition, these unknown ingredients inhibit proper crystal formation and growth. This results in impure sterols and lowers the rate of production.

The sterol content of the overhead fraction can be further increased to 30% to 35% by a conventional

FIG. 3. Sterols resulting from proper crystallization technique. 200 ×.

wet-refining procedure. This step, though desirable, is not essential.

Approximately half of the sterols in the overhead fraction are esterified with fatty acids. The next step is the saponification of the overhead fraction with 100% excess over theoretical caustic soda in methanol. Two volumes of 95% methanol are used for each volume of pitch overhead. Saponification occurs in 3 hrs. at gentle refluxing conditions. Hot water (160° F.) is slowly sprayed onto the surface of the batch until the volume is increased by 20%. Gentle refluxing is continued for another 2 hrs. The mixture is then cooled in 2 hrs. from 160°F. to 135°F. Gentle agitation is used throughout the saponification and chilling cycles. Care must be exercised to avoid sudden "shocking" of the batch either with cold reflux or with 20% water addition. If this care is not exercised, some of the soft and gummy unsaponifiable matter other than sterols precipitates. In addition, undesirable small crystals will result. The soft gummy material and the small crystals cause poor or negligible centrifuging and washing rates.

After chilling, the sterols are separated by using a perforated basket-type of centrifuge. When sufficient cake has been deposited, hot (140°F.) 95% methanol is sprayed into the cake and is continued until the effluent is colorless. Following the methanol wash, the cake is sprayed with hot water (180°F.) until the pH of the effluent is neutral. About 100 gals. of methanol and 100 gals. of water are required to wash 100 lbs. of sterols.

CONSIDERABLE experience and skill are required in the sequence of operations from the saponification to the final water-washing steps. With poor practice, precipitation of gummy material and small crystal formation result, and the resultant sterols are inadequate with regard to taste and color. The upgrading of a bad batch of sterols necessitates dissolving and recrystallizing in a solvent in which the sterols have an appreciably higher solubility than in methanol. Figures 3 and 4 show the difference in appearance of sterol crystals that result from proper and improper crystallization techniques.

After adequate washing the wet sterols are pelletized by using a modified meat grinder, placed on trays, and dried at 200°F. The dried sterols are packaged in fiber drums with a polyethylene inner lining.

The effluents from the centrifuge, through and including the methanol wash, are combined and then acidified. The methanol is recovered in a distillation column designed to produce 95% methanol overhead fraction. The still bottoms, excluding the water, has approximately 7–12% sterols, 25–30% other unsaponifiable matter, and 58–68% fatty and rosin acids, and a color of 37 FAC. Since these tall oil acids, containing a high percentage of unsaponifiable matter, are essentially free of oxidized and polymerized components, they may be distilled or used as a replacement for low-grade fatty acids.

The commercial plant was designed to produce 1,000 lbs. of sterols per day, requiring 11,000 lbs. of tall oil pitch per day. By-products amount to 5,500 lbs. per day of Solexol bottoms and 4,500 lbs. per day of tall oil acids with high unsaponifiable matter. Figure 5 shows a material balance of the process. Since the Solexol unit has a capacity considerably larger than required for the sterol plant, the Solexol

FIG. 4. Sterols resulting from improper crystallization technique. 200 ×.

unit in one day can produce a week's supply of feed material for the sterol plant.

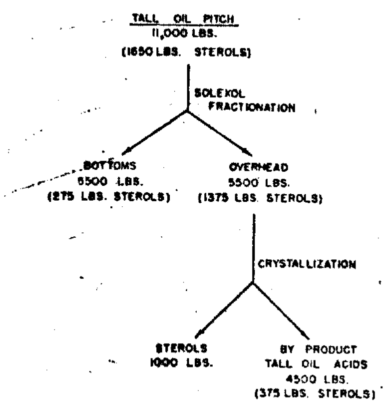

FIG. 5. Material balance.

With few exceptions all processing equipment is stainless steel. The acidulating tank is Monel, and the acid and caustic storage tanks are black iron. Every precaution is taken to remove dirt from the Solexol overhead fraction, methanol, caustic, and water used to charge the saponification reactor. Each of the charge lines for the above four streams is equipped with a filter. Once the overhead fraction and methanol come into contact, some of the sterols drop out of solution. Removal of dirt from this stage on becomes difficult. However magnetic particles are removed by a magnetic separator installed in the slurry line feeding the centrifuge.

The process described above uses a low cost by-product of the tall oil industry and has up-graded its value considerably. From tall oil pitch, pharmaceutical-grade sterols have been made. Thus a new commercial product has been added to the long list of products of the tall oil industry. Other large uses for tall oil sterols may develop, resulting in an increased demand for tall oil pitch. Considerable research is being conducted in steroid chemistry, such as cortisone, sex hormones, and many related compounds. The starting material for another medical triumph could be tall oil sterols.

Summary

A process has been developed for producing pharmaceutically-pure sterols from tall oil pitch. The process consists of the propane fractionation of the pitch, saponification of the overhead fraction in methanol, fractional crystallization, centrifuging, washing, and drying. A plant to produce 1,000 lbs. of sterols per day was brought into operation.

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The Effect of Fats and Fatty Acids in Chick Rations

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RECENT studies by Sielder and Schweigert (1953), Yacowitz (1953), Sunde (1954a), and Runnels (1955) indicated that the use of animal fats in poultry rations improved the utilization of the feed for broilers. Sunde (1954b) and Carver *et al.* (1954) reported that a hydrogenated fat or stearic acid would not improve feed utilization, but that all other fats tested would improve the utilization of the feed fed to young chicks. Both workers reported that the saturated fats were not absorbed by the digestive tract, and therefore offered very little nutritionally.

Biely and March (1954) reported that in both chick and poult rations the addition of fat may be advantageous when relatively high levels of protein are fed. Siedler *et al.* (1955) reported that 3 or 6 percent fat or 3 percent free fatty acids did not decrease the rate of gain and were utilized efficiently.

EXPERIMENTAL PROCEDURE

Day-old chicks of both sexes, the progeny of New Hampshire males and Single Comb White Leghorn females, were used except in experiments 6, 10 and 11. In experiment 6, New Hampshire chicks obtained from a commercial hatchery were used. In experiment 10, cross bred male chicks of the above cross were used. They were 31 days old when placed on experiment. In experiment 11, White Plymouth Rock chicks obtained from a commercial hatchery were used. Twenty-five chicks were used per group except where indicated. In experiments 6 and 11, twenty chicks were used per group. In experiments 7 and 8, the groups fed the linolenic, linoleic and butyric acid contained only 13 or 15 chicks because of the cost of these materials. All experiments were conducted in standard type electric batteries with raised wire floors. In experiments 9 to 11 all the birds had access to both types of feed in each battery at all times. When duplicate groups were set up, the diets were alternated on each side of the battery. This would eliminate the light factor, and also the bird's desire to be near or away from the most activity. One feeder contained the added fat, and the other the feed with no added fat. The deck immediately below that one had the two types of feed reversed. Once during the experimental period the feeders were switched to see how long it took the birds to adjust to the new conditions. The basal diets fed in all experiments are shown in Table 1. Additions were made in such a way as to keep the protein level constant. This was done by decreasing the corn and increasing the soybean oil meal.

Tenox II* was added at the level of .05 percent to all the fats and fatty acids used except the stearic acid and the hydrogenated fats. The time required for feed

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* This mixture contains 20 percent butylated hydroxyanisole, 6 percent propyl gallate, 4 percent citric acid, and 70 percent propylene glycol.

1.—*Basal diets*

	A (gms./kg.)	B
Ground yellow corn	450	555
Soybean oil meal (solvent)	300	350
Wheat bran	50	
Wheat middlings	50	
Alfalfa meal	50	30
Steamed bone meal	30	
Ground limestone	—	12.5
Di-calcium phosphate	—	17.5
Vitamin D_3 (1,500 D/gm.)	—	.5
Granite grit	10	
Chick size oystershell	20	
Iodized salt	5	2.5
Feeding oil (300 D-1,500A)	2	
Fish solubles	30	30
Vitamin B_{12} and antibiotic feed supplement*	1	1
DL-Methionine	0.5	0.5
$MnSO_4 \cdot H_2O$	0.22	.25
Ca. pantothenate mgs./kg.	5	22
Niacin mgs./kg.	10	35
Riboflavin mgs./kg.	3.2	7

To Diet B were added the following vitamins in mg. per Kg. of ration: thiamine HCl 4, pyridoxine HCl 7, d biotin .2, menadione .8, i-inositol 1,000, para-amino-benzoic acid 100, alpha tocopherol acetate 5, and folic acid 2.

* Each pound equivalent to the activity of not less than 3 milligrams of vitamin B_{12} (L. L. elution assay) and 2 grams procaine penicillin.

passage was determined by feeding 500 milligrams of carmine in a zero size capsule and determining the time required for the red dye to be voided. The chicks were four weeks old when this test was performed. Ten birds from each group were used for each measurement.

The crude tall oil† material contains about 41–52 percent rosin acids and 46–52 percent fatty acids. It is a product obtained from woodpulp manufacturing especially of pine. About 180–300 pounds of this material are produced per ton of woodpulp. The tall oil is a distilled product obtained from the crude tall oil. It contains about 61–71 percent fatty acids and 25–30 percent rosin acids. The fatty acid fraction contains about 45 percent oleic acid, 48 percent linoleic acid, and 6–7 percent palmitic acid. The rosin acid fraction was concentrated from the crude tall oil by removing as much as possible of the tall oil. The crystalline materials contain about 90 percent rosin acids. The rosin acid fraction is composed of about 30–40 percent abietic acid and 10–20 percent neoabietic acid. Other rosin acids are also present in smaller amounts.

† Ligro produced by the West Virginia Pulp and Paper Company, Charleston, South Carolina.

TABLE 2.—*The effect of different grades of fat on growth and feed conversion*

Experiment 1

Additions to basal A	4 wk. wts. (gms.)	Gm. feed / Gm. wt. 4 wks.	Time of feed passage (minutes)	
None	292	2.02	146	132
None	288	2.17		
5% White grease	324	1.79		
5% Yellow grease	313	1.95		
5% Brown grease	298	1.93		
5% Prime tallow	321	1.97	126	144
2.5% Prime tallow	300	1.96		
5% No. 1 tallow	322	1.93		
5% Commercially stabilized fat*	293	1.94		
5% Hydrogenated fat†	289	2.03		

* Sta-fat—Darling Manufacturing Co., Chicago, Illinois.
† Hydropoid—Bowman Feed Products, Inc, Holland, Michigan.

The feces samples were collected on the second and forth week as indicated in the tables. The samples were weighed and the fat and free fatty acid analyses conducted according to the method of Saxon as described by Hawk, Oser and Summerson (1947).

RESULTS AND DISCUSSION

The results of the first experiment are shown in Table 2. The addition of five percent choice white grease, yellow grease, brown grease, prime tallow, No. 1 tallow, a commerically stabilized fat or hydrogenated fat‡ did not decrease the growth rate. In most instances an increase in the rate of growth was observed. All the supplements to the basal diet except the hydrogenated fat improved the feed utilization (gms. feed/gm. weight). These

‡ Hydropoid-Bowman Feed Products, Inc., Holland, Michigan.

TABLE 3.—*The effect of different grades of fat on growth and feed conversion*

Experiment 2

Additions to basal A	4 wk. wt. (gms.)	Gms. feed / Gm. wt. (4 wk.)
None	305	2.04
None	313	2.08
5% White grease	297	1.90
5% Yellow grease	302	1.96
5% Brown grease	294	2.02
5% Prime tallow	289	2.07
5% No. 1 tallow	297	1.91
5% Commercially stabilized fat*	311	1.89
5* Hydrogenated fat†	311	2.05
2.5% Hydrogenated fat†	305	2.18

* Sta-fat—Darling Manufacturing Co., Chicago, Illinois.
† Hydropoid—Bowman Feed Products, Inc., Holland, Michigan.

differences were repeated in other experiments. The possibility existed that the fat might decrease the rate of the passage of the feed through the intestinal tract. Munson *et al.* (1950) reported that dextrinized corn starch increased the time of excretion over that observed when either sucrose or lactose was used as the carbohydrate in rations for chicks. The added prime tallow did not increase the time required for the passage of the feed through the tract (Table 2). This extremely rapid passage of food through the young chick emphasizes even further the importance in poultry feeding of having all the essential nutrients present in proper amounts at all times.

Table 3 shows the data of another experiment conducted in about the same manner as experiment 1. The prime tallow was tested only at the 5 percent level, but the hydrogenated fat which was ineffective in improving feed utilization in experiment 1 was tested at both the 2½ and 5 percent levels. Again it did not decrease the growth rate, but was ineffective at either level at improving feed utilization. All other supplements except the prime tallow improved feed utilization in this experiment. In other experiments not reported here prime tallow has been effective in improving feed utilization. Aitken, Lindblad and Hunsaker (1955) also reported on the effects of tallow on feed efficiency. These experiments show that these grades of fats can be used effectively except the hydrogenated fat. This has been reported previously by Sunde (1954b), Carver *et al.* (1954), Donaldson, Combs and Romoser (1954), and Siedler, Scheid and Schweigert (1955). Five or 2½ percent fat was used in these experiments. The level of fat that will be most economical will depend on the price of the fat and the price of the grains. Yacowitz and Chamberlin (1954) fed levels from 1.5 to 3.0 percent. On floor litter 1.5 percent fat improved feed efficiency 7 percent as compared to 3.1 percent with 3 percent fat. In batteries, however, 3 percent was better than 1.5 percent. The work from this laboratory has suggested that levels of fat of as high as 10 to 22 percent (Sunde, 1955; Leong *et al.*, 1955) increased the feed efficiency even further. Combs and Romoser (1955) have also fed 15 percent with an increase in efficiency.

Table 4 shows the results of feeding 5 and 10 percent white grease, 5 percent oleic acid and 5 percent hydrogenated fat. None of the supplements decreased the

TABLE 4.—*The effect of fats and fatty acids on body weight, feed conversion, feces fat and free fatty acids in the feces of chicks*

Experiment 3

Additions to basal A	4 wk. wts.	Gms. feed / Gm. wt.	% fat in dry feces	% FFA of feces fat
None	283	2.14	1.18	35
None	325	2.09		
5% white grease	311	1.92	3.17	64
10% white grease	289	1.91	5.80	59
5% Oleic acid	292	1.96	3.68	67
5% Hydrogenated fat†	309	2.21	9.27	82

† Hydropoid—Bowman Feed Products, Inc., Holland, Michigan.

growth rate. Again the addition of 5 percent hydrogenated fat failed to improve the utilization of the feed. All other supplements improved this utilization. An attempt was made to determine the digestibility of the fat in the feed. The addition of the white grease increased the amount of fat in the dry feces by 2 to 5 times depending upon the level. This was slightly lower in subsequent experiments. Five percent oleic acid increased the fat in the feces about the same as the 5 percent white grease. The amount of fat in the feces resulting from the feeding of the hydrogenated fat increased about 8 to 9 times over that of the basal diet. This indicates that the fat was not utilized to any appreciable extent. An attempt was made to determine the amount of the ether soluble fraction that was present as free fatty acids. The feces from the group fed the hydrogenated fat contained 82 percent free fatty acids. This suggests that the glycerol portion of the molecule was removed and that the free fatty acids were not absorbed. The determination employs the milliequivalents of sodium used to titrate to the phenolphthalein end point and uses the 18 carbon fatty acid as a basis to determine the amount of free fatty acid present. Either the chicken has the ability to remove the glycerol portion of the fat and not the saturated fatty acid or the fat is broken down into smaller fragments which have acid groups which can be titrated.

TABLE 5.—*The effect of fats and fatty acids on body weight, feed conversion, feces fat and free fatty acids in the feces of chicks*

Experiment 4

Additions to basal A	4 wk. wts.	Gms. feed / Gm. wt.	% fat* in dry feces
None	326	1.90	.44
5% white grease	336	1.89	1.96
5% Stearic acid	324	2.03	7.68
5% Oleic acid	326	1.77	1.78

* 2 week data.

TABLE 6.—*The effect of dispersing agents on body weight, feed conversion and feces fat of chicks*

Experiment 5

Additions to basal A	4 wk. wts.	Gm. feed / Gm. wt.	% fat in dry feces
None	330	2.05	2.63
5% white grease	350	1.77	1.57
.2% Sodium ligno-sulfonate*	342	2.07	1.00
.2% Sodium lingo-sulfonate* and white grease	358	1.98	3.18
5% Stearic acid	318	2.19	9.01
5% Stearic acid and sodium ligno-sulfonate	326	2.19	9.01
5% Stearic acid and .02% surfactant†	325	2.16	7.76
Practical broiler mash	345	2.01	1.76

* Marasperse N from Marathon Corporation, Rothschild, Wisconsin.

† Ethomid HT 25 from Armour Laboratories, Chicago, Illinois.

In experiment 4 (Table 5) stearic acid as well as oleic acid and white grease were included in the experimental design. This was done to determine the effect on absorption of the completely saturated fat. Again weights were not affected but feed utilization was not improved with stearic acid. The basal group was more efficient than average (Tables 2, 3, and 4). Oleic acid was especially effective in this experiment. The percent fat in the feces is about the same for the oleic acid as for the white grease, however, the stearic acid was not absorbed to any extent from the intestine.

Table 6 shows the results of an attempt to use a dispersing agent to improve the utilization of the stearic acid. Sodium ligno-sulfonate did not decrease the percent fat in the feces when added to a diet containing either white grease or stearic acid. The use of a surfactant was not very effective either. The only group to show any improvement in feed utilization was the one fed the white grease alone. Whether the sodium ligno-sulfonate actually decreased the feed efficiency is doubtful since no effect one way or the other was exerted in any group except the one

TABLE 7.—*Effect of varying the energy in the basal diet on feed conversion and body weight at 10 weeks*

Experiment 6

	Wt. (gms.) M	Wt. (gms.) F	Gms. feed / Gm. wt.
Basal diet (A)	1,515	1,367	3.01
Basal diet+5% white grease	1,700	1,366	2.60
High energy basal (Diet B)	1,580	1,308	2.75
High energy basal+5% white grease	1,643	1,399	2.38

containing the white grease. Thus it appears these surface active materials do not increase the birds ability to remove long chain saturated fatty acids from the intestine.

The data presented here and the previous data from our laboratory were obtained using a diet of medium energy content. Many other laboratories have used higher energy diets. Table 8 shows the results of an experiment making a comparison between the two types of diets. The high energy diet was made by using largely corn and soybean oil meal with low levels of alfalfa meal and supplementing this diet with minerals and vitamins. Supplementing either diet with five percent white grease improved the growth rate slightly at 10 weeks. This has been true in several of our trials to 10 weeks, Sunde (1954a). The 10 weeks' data of Pepper *et al.* (1953), Siedler *et al.* (1955) and Runnels (1955) also suggest this same effect. It is of interest that the addition of white grease to either type diet resulted in a similar improvement in feed utilization. The improvement was .41 pound of feed in one instance and .37 in the other. Perhaps the protein level was a bit more than adequate for each energy level.

TABLE 8.—*The effect of fatty acids on body weight and feed conversion in chicks*

Experiment 7

	Wt. (gms.)	Gms. feed / Gm. wt.
Basal diet A	280	2.21
Basal+5% oleic acid	276	1.95
Basal+5% stearic acid	294	2.08
Basal+5% linolenic acid	281	1.99
Basal+5% linoleic acid	275	1.96
Basal+5% butyric acid	258	2.00
Basal+5% white grease	277	1.88

TABLE 9.—*The effect on chicks of tall oil products, and fatty acids on body weight, feed conversion and feces fat*

Experiment 8

	Wt. (gms.)	Gms. feed / Gm. wt.	% fat in dry feces
Basal diet A	302.2	2.04	1.12
	312.5	2.05	1.20
Basal+5% crude tall oil	155.8	3.05	—
Basal+5% tall oil	201.7	3.01	2.44
Basal+5% rosin acids	88.9	—	—
Basal+5% oleic acid	282.7	1.95	1.89
Basal+5% butyric acid	204.7	1.95	.94
Basal+5% linoleic acid	316.3	1.91	1.45
Basal+5% linolenic acid	327.3	1.89	2.11
Basal+5% white grease	290.1	1.92	1.27

Tables 8 and 9 show the results of feeding several fatty acids to chicks. Butyric was included to see what use the chickens would make of this short chain fatty acid. Butyric acid depressed growth in both experiments. Feed utilization was improved with this material. Oleic acid, linolenic or linoleic acid improved the feed utilization and did not affect growth.

When the crude tall oil was fed, the growth of the chicks was depressed when compared to groups receiving oleic, linolenic or linoleic acid. Tall oil reduced the growth rate in spite of its high free fatty acid content. The cause of this reduction is probably due to its rosin acid content. When the rosin acids fraction was fed at the five percent level, growth was depressed markedly. The growth depressing effect of the crude tall oil was intermediate between the high fatty acid fraction and the high rosin acid fraction. All three of these products reduced feed utilization probably because of their depressing effect on growth. It is of interest to notice that the percent fat in the dry feces is as high as with oleic acid. No determinations of fat were made on the feces of the rosin

TABLE 10.—*The results of cafeteria feeding of feed with and without added fat to chicks*

	Feed consumed in grams				Consumption ratio	
	Without fat		With fat			
	A	B	A¹	B¹	A¹/A	B¹/B
Experiment 9						
Time						
0–7 days	380	385	545	705	1:1.4	1:1.8
0–14 days	1,100	1,045	2,367	3,048	1:2.15	1:2.9
0–21 days	2,315	2,720	5,380	6,260	1:2.3	1:2.3
0–28 days	4,950	4,595	9,610	10,285	1:1.9	1:2.2
Experiment 10						
31–35 days	315	562	1,972	1,990	1:6.3	1:3.5
31–41 days	1,825	983	3,585	3,747	1:2.0	1:3.8
31–48 days	2,140	1,545	5,557	5,737	1:2.6	1:3.7
Experiment 11						
0–7 days	500		1,100		1:2.2	
0–10 days	965		1,930		1:2.0	
0–17 days	1,820		3,825		1:2.1	
0–22 days	2,640		5,000		1:1.9	

acid fed chicks or those fed the crude tall oil because of the severe growth depressing effect of those materials. If the rosin acids could be removed from these materials efficiently, this product might find a use in poultry feeds. At the present time the price of the distilled oil is about 6½ cents per pound in tank car lots.

The data on the fatty acids tested shows that the free fatty acids themselves are not harmful and that the free fatty acid content of an animal fat is not important. The free fatty acid content of a material, however, may give some indication as to the past history of the material, and therefore may be of value to the purchaser of inedible animal fats. This is true not because of the free fatty acid content, but because of other break down products it may contain.

Table 10 shows the results of three experiments set up in such a way as to obtain data on whether or not the birds prefer feed with added fat. The groups were set up in duplicate in all but experiment 11. The data show that light or general activity were not important since feed consumption figures of the duplicate groups were about the same. The groups were distributed in such a way as to eliminate this as a variable. In all groups, the birds preferred the feed with the added fat. When given access to both types of feed, they consumed about two times as much of the feed containing added fat as the feed with no added fat. This relationship held both for the chicks started at one day of age and at 31 days of age. The chicks started at 31 days of age had been on another experiment until the 28th day. No added fat was included in any of the rations used up until the 31st day. Thus it appears that battery chicks, at least, prefer feed with added fat when they have a choice. This does not mean that they will eat more of a feed with added fat when given only one feed, but rather that when given a choice they prefer the one with the added fat. We do not know if texture or color is the more important factor.

SUMMARY

Representative types of inedible animal fats have been fed to chicks. Choice white grease, brown grease, prime tallow and No. 1 tallow were used in chick starting diets without deleterious effects at the five percent level. All these materials improved feed utilization. Oleic acid, linolenic and linoleic acid did not affect the growth rate and improved feed utilization. The incorporation of five percent hydrogenated fat or stearic acid in the diet did not improve the feed utilization. Apparently the chicks did not utilize the saturated long chain fatty acids provided by these materials.

A comparison of a medium and a high energy formula was made and the addition of fat to either diet resulted in about the same improvement in feed conversion. Fatty acid fractions of crude tall oil preparations were toxic to the chicks probably because of the rosin acids which they contained.

Chicks given access to feed with and without added fat ate about twice as much of the feed with the added fat.

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THE EFFECT OF KRAFT PULP MILL WASTES ON SOME AQUATIC ORGANISMS

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ABSTRACT

A study has been made of the toxic substances which may be found in kraft pulp-mill waste-waters. It has been determined that the sulphides, mercaptans, resin acid soaps, and sodium hydroxide constitute the greatest hazard. The minimum lethal concentration of these and other materials to fresh-water minnows, *Daphnia*, and aquatic insect larvae has been established. Methods have been devised for evaluating these materials in kraft-waste waters, and data from the examination of the wastes of a typical northern kraft mill are presented.

INTRODUCTION

The pulp and paper industry has for years been interested in problems relating to stream improvement since large amounts of water are required in the making of its products. This water is not a part of the final product but is used, among other ways, as a medium for pulp refining and sheet formation. During its use, solids are added to it, either in solution or suspension, and when, after its use, the water is returned to the stream, these solids can have an adverse effect on the stream.

There are at least three ways by which any industrial or domestic waste can affect the aquatic environment. First, the waste may contain toxic substances which will destroy the fauna and/or flora; second, it may contain materials in solution, the stabilization of which uses so much oxygen that the aquatic forms are adversely affected; and third, there may be a combination of these two types of action.

In the current study, attention has been directed to the first of these three possibilities in relation to kraft mill operation. Until recently this problem has received comparatively little attention. Cole (1935) investigated the effects of kraft black liquor on perch, bluegills, largemouth black bass, and rock bass, and found that the material was harmless in concentrations up to 5,000 p.p.m. In Scandinavia, Bergström and Vallin (1937) and Bergström (1939) studied the effect on fish of various components of kraft mill effluents, including digester condensates, dilute wash waters, and other wastes. He found that, of all the sources of waste arising from the kraft process, those containing the sulphate soap carried the greatest potential danger. This same fact had been reported earlier by Ebeling (1931). Hagman (1936) found that resin

[1]Environmental Health Center, U. S. Public Health Service, Cincinnati, Ohio.

acids in sufficient concentrations would harm fish, and stated that the safe concentration of these acids was 2 p.p.m. or less. Extrom and Farner (1943) concluded that bass *(Huro salmoides)*, bluegills *(Lepomis macrochirus)*, and sunfish *(Lepomis gibbosus)* were unaffected by "river concentrations" of kraft mill wastes although the fish could not live in the wastes themselves.

The inconclusive nature of the work cited above, together with the need for more specific information, impelled segments of the kraft pulping industry to conduct and support an investigation designed to clarify the entire relationship between the kraft mill and the stream. Accordingly, the work started by Cole and continued by Extrom and Farner was brought to The Institute of Paper Chemistry in 1943. At the Institute, the study was first supported by the Committee on Kraft Waste Disposal, a group representing various kraft mills in Wisconsin. For the past 5 years, however, the investigation has been supported and extended by the National Council for Stream Improvement, Inc. (an organization of pulp and paper companies) whose purpose is to study this and other pollution problems in the pulp and paper industry. The objectives of the present investigation may be listed as follows: (1) to determine if and to what extent the waste liquors from a kraft mill are toxic to aquatic organisms; (2) to devise methods for the detection and measurement of these toxic components; and (3) to discover measures which will prevent these wastes from reaching the streams.

There are several ways by which chemical wood pulp can be manufactured, among which are the sulphite process, the kraft or sulphate process and, more recently, the semichemical process. Of these the kraft method is the most widely used, with the sulphite process second in importance. In the sulphite process the liquor used for digesting the wood is an aqueous solution of sulphurous acid in which lime or some other base has been dissolved. The result is, therefore, a solution of a bisulphite of the base containing an excess of sulphurous acid. When calcium[1] is used as the base, the nature of this liquor, as well as the mechanics of the sulphite process, makes it economically unfeasible to recover the spent liquor. It is, therefore, wasted and is commonly known as sulphite waste liquor.

In the kraft process, on the other hand, the wood is cooked or digested with an alkaline liquor in which the principal components are sodium hydroxide and sodium sulphide, the latter comprising up to 45 percent of the total alkali. From the point of view of the problem at hand, the important aspect of the kraft process is that instead of being wasted the spent liquor is evaporated and burned, and the chemicals in the ash are re-used and the heat is recovered.

[1] In recent years the Weyerhaeuser Timber Company has developed an acid cooking process in which magnesium instead of calcium is used. In theory, this process permits the recovery of the chemicals and burning of the residue for heat recovery, thus materially reducing, if not eliminating, the problem of stream pollution. However, this development is still in the experimental stage.

In order to delineate more clearly the problem of stream pollution which may result from a kraft mill, it is desirable to consider briefly the general aspects of the process. The wood is cut into small chips and introduced into digesters, the cooking liquor is added, and the digester closed. Heat is then applied gradually, by direct or indirect steaming, and the cooking process is started.

The cooking cycle of any type of chemical pulp production consists of three phases: (1) a period when the liquor is permeating the chips, (2) a period of full pressure when the cellulose fibers are being freed from their bonding material, and (3) a gassing-down period after the cook when the digester's internal pressure is gradually relieved to the point where the pulp can be blown out. At the conclusion of the first phase, the accumulated air and other gases in the digester are drawn off and passed through a condenser for heat recovery. Turpentine is recovered from the condensate, and the residue becomes waste. Similarly, at the conclusion of the second phase, the relief gases are condensed and may become waste.

After the pulp is blown from the digester, the spent cooking liquor (black liquor) is separated from it and sent to the recovery plant. The pulp is washed with water that is re-used until the black liquor concentration is high enough for wash waters to be sent to the recovery plant. In the final washing process, however, the waters may be so dilute that their chemicals cannot be recovered economically and, therefore, they may be sent to the sewer.

The spent cooking liquor and the concentrated wash waters are evaporated under vacuum to a consistency of approximately 50 percent solids. In most mills this vacuum is produced by a jet condenser and its effluent may contain in solution some of the non-condensable gases from the process of black liquor evaporation. This effluent may be sent to the sewer or, in some mills, it is used for pulp washing and eventually finds its way back to the recovery plant. Use of surface condensers instead of the jet type will reduce, if not eliminate, the possibility of pollution from this source. The concentrated black liquor, containing most of the non-fibrous portion of the original wood as well as the spent chemicals, is then burned in furnaces designed to recover the chemicals and the heat of combustion.

When the black liquor from the blowpits (into which it is blown from the digester) is sent to the recovery plant, it is usually stored in large receptacles for varying periods. As the material stands, a thick foamy material, called sulphate soap, rises to the surface. Actually, it is composed of the sodium salts of resin and fatty acids which were present in the wood. Currently, this soap is being recovered and sold, but small amounts of it may be found in the dilute wash water.

In summary, it can be stated that the major potential stream polluting products from the normal operation of a kraft mill are: (1) the initial blowdown condensate, (2) the final blowdown condensate, (3) the evaporator condensate, and (4) the soaps and other material in the dilute wash waters.

Method of Testing Toxicity of Wastes

According to Sutermeister (1941), kraft waste liquors may contain the following compounds: sodium carbonate, sodium hydroxide, sodium sulphide, sodium sulphate, sodium sulphite, sodium chloride, hydrogen sulphide, methyl mercaptan, and sodium combined with organic acids, such as the sodium salts of resin and fatty acids (soaps). It is apparent that any of these compounds might prove toxic if they are present in sufficient amounts. The first problem, therefore, was to determine the minimum lethal concentration of these materials to various aquatic species.

In choosing test animals, the more susceptible species of each group were selected. A secondary consideration was the environmental relationship of the groups, i.e., fish and fish-food organisms. The fish used were species of minnows taken from various sources in the vicinity of Appleton, Wisconsin. Lake Emerald *(Notropis a. atherinoides)* and spotfin *(Notropis spilopterus)* shiners were most commonly used but, at times, the bluntnose minnow *(Hyborhyncus notatus)* and the rosyface shiner *(Notropis rubellus)* were also used. Two types of fish-food organisms were used as test animals. The first of these was *Daphnia sp.* grown in the laboratory for the purpose, and the second was aquatic insect larvae of various species taken from Lake Winnebago and adjacent waters.

The method using minnows as test animals was a modification of that employed by Powers (1917) and has been previously described by the senior author (Van Horn, 1943). Two-liter portions of the materials to be tested were placed in open battery jars immersed in a constant temperature (18° C.) water bath. From one to five fish, depending on the oxygen resources of the test solution, were then placed in each jar, and observations were made hourly up to 5 days. The survival time of the fish was recorded if death resulted. Checks were made on the dissolved-oxygen content of the test solution at the conclusion of the test and, in those cases where this level fell below 4 p.p.m., the data were discarded. Checks were also made on pH and alkalinity to make sure that the conditions were within limits favorable to fish life. If these conditions fell outside of these limits, which was very seldom, the results were discarded.

When *Daphnia* was used as the test animal, two specimens were placed in a vial containing 25 milliliters of the test solution. The vials were held in racks, each of which carried 26 test vials and 6 controls. Periodic observations were made for a period of 48 hours, and the length of time elapsing between the time of placing the specimens in the

solution and immobilization was recorded. Checks on dissolved oxygen, pH, and alkalinity were made before and after each test.

When aquatic insect larvae were employed as test animals, the method used for *Daphnia* was modified to accommodate the physiological requirements of the insect larvae. The larvae were placed in test solutions contained in glass vessels of suitable size and were permitted to remain there for a period of 48 hours. In the meantime, a close watch was kept on them. Extraneous variables, such as pH, alkalinity, and dissolved oxygen, were checked as described above. The species used in these studies were taken from Lake Winnebago and adjacent waters and included mayflies of the genus *Blasturus* and *Leptophlebia*, the damsel fly *Agrion*, and the dipteran *Chironomus*. All tests were made using stabilized Fox River water. This water is relatively hard, having a pH of 7.6 to 7.8 and a total alkalinity of from 140 to 160 p.p.m.

Toxicity of Kraft Mill Wastes

In order to determine which of the components of a typical kraft mill waste would be significant in this study, a number of screening tests were made on 15 compounds, using the Lake Emerald and spotfin shiners as test animals (Table 1). It is apparent that the compounds of greatest

Table 1.—*Minimum lethal concentrations*[1] *of kraft pulp-mill waste components killing Lake Emerald and spotfin shiners in 120 hours.*

Compound	Concentration, p.p.m.
Sodium hydroxide	100.0
Sodium sulphide	3.0
Methyl mercaptan	0.5
Hydrogen sulphide	1.0
Sodium thiosulphate	5.0
Sodium chloride	2,500.0
Sodium carbonate	250.0
Sodium sulphate	100.0
Formaldehyde	50.0
Crude sulphate soap	5.0
Sodium salt of fatty acid fraction of crude soap	5.0
Sodium salt of resin acid fraction of crude soap	1.0
Sodium oleate (stock room)	5.0
Phytosterol (pure)	3.0
Sodium abietate (pure)	3.0

[1]In this work the "minimum lethal concentration" was defined as the lowest concentration of a toxic material which would kill any of the test animals within a period of 120 hours, when held in a test solution at 18° C., and factors of pH, total alkalinity, and dissolved oxygen being held to conditions approximating those of control tests. 100 percent survival of controls was required.

interest in this study are sodium hydroxide, the sulphides, methyl mercaptan, and the crude sulphate soap and its components. Furthermore, under normal operating conditions, kraft waste waters are relatively constant in their composition and in the proportion of one component to another. For these reasons, particular attention was directed to the four compounds indicated, and an extensive series of tests, in addition to those just described for minnows, was made on *Daphnia* and two species of aquatic insect larvae.

The data contained in Table 2 indicate that the minimum lethal concentration was roughly the same for the species of minnows tested, *Daphnia*, and the mayfly larvae. While mayfly larvae were less sensitive to soap (10 p.p.m.), they were more sensitive than either of the other forms to sodium sulphide. The midge larvae (*Chironomus* sp.) are much less sensitive to all the toxic compounds tested.

TABLE 2.—*The minimum lethal concentrations of kraft pulp waste components affecting minnows,[1] Daphnia, and two species of aquatic insect larvae.[2]*

Compound	Minimum Lethal Concentration, p.p.m.			
	Minnows	*Daphnia*	Mayfly Larvae[2]	*Chironomus* Larvae
Sodium hydroxide	100.0	100.0	100.0	700.0
Sodium sulphide	3.0	10.0	1.0	1,000.0
Hydrogen sulphide	1.0	1.0	1.0	750.0
Methyl mercaptan	0.5	1.0	1.0	50.0
Crude sulphate soap	5.0	5.0	10.0	50.0
Sodium salt of resin acid fraction of soap	1.0	3.0	....	50.0
Sodium salt of fatty acid fraction of soap	5.0	1.0	....	....

[1]The spotfin shiner *(Notropis spilopterus)* and the Lake Emerald shiner *(Notropis a. atherinoides)*.
[2]Mayfly larvae used were of the genera *Blasturus* and *Leptophlebia*. No attempt was made to determine species.

QUANTITY OF TOXIC MATERIALS IN WASTE WATER

Having established the maximum safe concentration of the potentially toxic materials found in kraft pulping wastes, the next step was to measure the amount passing to the streams. The methods of analysis adopted are being reported in detail elsewhere but they may be briefly described here.

It was apparent that the various sulphides need not be determined individually and, for the sake of convenience, it would be better if the sulphides could be collectively evaluated by a single analysis. Tamele and Ryland (1936), Tamele, *et al.* (1941), Lykken and Tuemmler (1942), and Borlew and Pascoe (1946) have described such a method which involves the use of a potentiometric-argentimetric titration in a highly alkaline solution. The applicability of this method was thoroughly explored, and it was determined that it would be effective in the current problem. Furthermore, if chlorides are not present in any great amount, it is possible to use the same titration for mercaptan evaluation. For the resin acids, an adaptation of the Liebermann reaction was used. The sodium hydroxide analysis consisted of a simple potentiometric titration with a weak acid to a pH of 8.4.

During the period from May to September, 1947, the waste waters of five typical northern kraft mills were subjected to frequent analyses for sulphides, mercaptans, resin soaps, sodium hydroxide, and pH. Care

was taken to sample under all types of operating conditions. All samples taken were grab samples. It should be noted that 24-hour composite samples of these wastes will not show extremes in variation of their chemical content. It has been demonstrated, for example, that, if a sample having a high pH is permitted to stand for an hour or so, its pH will gradually change in the direction of neutrality. For that reason, grab samples were taken to determine the degree of variation in the nature of the waste waters. At the time the samples were taken, data on the volume of sewer discharge and the volume of river discharge were secured. A typical analysis for one mill is shown in Table 3.

It will be noted from Table 3 that, in 13 of the 35 analyses of raw waste, the concentration of sulphides in the waste water exceeded the minimum lethal concentration (1 p.p.m.); in all cases, the concentration of mercaptans exceeds the minimum lethal concentration (0.5 p.p.m.); in all cases, content of the resin acid soaps exceeded the minimum lethal concentration (1 p.p.m.); and, in 4 of the 35 analyses, the concentration

TABLE 3.—*The results of waste water analyses of a typical northern kraft mill. June 24 to September 12, 1947.*

Sample No.	River dilution ratio[1]	Parts per Million				
		Sulphides	Mercaptans	Resin soaps	Sodium hydroxide	pH
1	170	0.7	1.5	5.0	6.6	8.6
2	166	0.6	0.7	5.0	8.0	8.8
3	178	1.5	4.1	18.0	61.4	9.9
4	218	1.6	1.8	8.0	24.2	9.4
5	185	1.0	1.2	5.0	12.0	9.0
6	193	0.2	1.9	5.0	0.0	7.8
7	275	18.4	12.0	18.0	114.0	10.6
8	232	0.5	2.5	3.0	6.6	8.7
9	224	0.2	0.7	3.0	0.0	8.2
10	138	0.4	0.9	2.0	0.0	8.2
11	142	0.9	1.6	3.0	0.0	8.2
12	126	0.0	1.0	3.0	0.0	8.1
13	123	0.8	1.6	3.0	14.7	8.7
14	193	0.0	2.7	5.0	10.6	8.7
15	173	0.2	1.4	5.0	0.0	8.3
16	191	0.2	0.9	5.0	14.7	8.8
17	173	0.2	1.2	2.0	0.0	7.6
18	176	0.0	1.1	5.0	0.0	7.9
19	166	0.0	1.2	3.0	0.0	7.9
20	177	0.2	1.1	3.0	0.0	7.7
21	153	0.2	1.1	3.0	0.0	8.4
22	152	0.0	0.8	3.0	0.0	8.1
23	157	0.0	0.6	2.0	0.0	8.3
24	144	0.3	1.0	2.0	2.6	8.5
25	156	2.1	1.9	3.0	13.3	8.9
26	161	88.4	15.8	18.0	391.0	10.5
27	113	0.4	1.6	3.0	4.0	8.5
28	103	0.3	1.7	3.0	17.3	9.0
29	96	0.0	3.1	5.1	9.3	8.7
30	94	2.8	3.3	8.0	21.4	8.8
31	94	25.8	8.1	18.0	115.0	9.4
32	134	0.4	1.0	3.0	144.0	10.7
33	136	3.6	5.1	5.0	13.7	8.9
34	127	2.8	7.2	3.0	9.4	8.8
35	109	1.3	0.9	5.0	0.2	8.5

[1]The river dilution ratio is calculated by dividing the volume of river discharge by the volume of sewer discharge.

of sodium hydroxide exceeded the minimum lethal concentration (100 p.p.m.). As soon as the wastes are discharged, dilution with river water takes place at varying rates depending on the volume of river flow. The lowest dilution ratio observed at this particular mill was 1 to 94 (Table 3). If total dilution is assumed, the concentrations of the toxic materials would be brought to a point below their minimum lethal concentration as soon as the necessary dilution had occurred.

In the entire study, including phases not presented here, the lowest ratio of dilution found in the two receiving streams was 1 to 73. Similarly, the highest concentrations of the compounds under study were sulphides 88.4 p.p.m., mercaptans 45 p.p.m., resin acid soaps 28 p.p.m., and sodium hydroxide 3,763 p.p.m. Although these conditions did not occur simultaneously, they do form a theoretical picture of what could happen under the worst conditions in the mills under study. In each case, however, the concentrations of the materials after dilution are brought to a point where their danger is reduced to a minimum, if not eliminated.

Discussion

The primary purpose of this investigation was to determine whether or not a problem relating to aquatic organism toxicity is associated with the normal operation of a kraft pulp mill and, if there is a problem, to determine its seriousness. From the data presented it can be stated that, under normal operating conditions in the locality where the study was made, there is little danger to the aquatic environment from the toxic properties of kraft pulp-mill wastes. While the majority of analyses of waste waters indicated that they contained toxic materials in excess of their minimum lethal concentration, their concentration was reduced to a level safe for fish and other forms after dilution in the stream. It should be pointed out also that, when fish or other aquatic organisms come into contact with these materials in lower concentrations, death does not occur instantly. For example, in a concentration of methyl mercaptan of 1 p.p.m. (twice the minimum lethal concentration), fish may live for as long as 2 days before exhibiting any of the reactions of poisoning.

If a kraft pulp mill is operating normally, there seems little danger that it may be responsible for destroying an aquatic environment by the toxic action of its wastes, providing enough receiving water is available for sufficient dilution. Abnormal or accidental incidents may sometimes occur in the operation of any industrial installation. For that reason, this investigation is being continued to determine at which step in the operation the potentially toxic materials occur, and to find ways and means of preventing their passage to the stream in the event of accidents or other abnormal conditions.

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152

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Experimental Carcinogenicity and Acute Toxicity Of Representative Epoxides

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Carcinogenic and acute toxicity potential were assayed while more than 60 epoxy compounds were being studied for commercial utility. Range-finding toxicity data are presented for 60; lifetime mouse skin painting results are given for 28, and mouse sebaceous gland suppression results are listed for 26 of these compounds. None of the 11 monoepoxides produced tumors while 5 of the 17 diepoxides were tumorigenic during lifetime skin painting of mice. The median latent period for tumor production with the diepoxides was 15 to 23 months. No generalities about the toxic, irritative or carcinogenic hazards are justified by the presently known facts. Each compound must be individually studied to determine toxicity and carcinogenicity potential.

Introduction

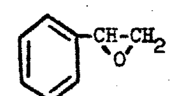
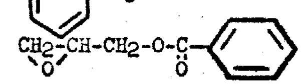
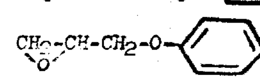
ALTHOUGH commercial utilization of epoxy compounds is relatively new, in recent years the hazards to health of utilization of the epoxy monomers and resins have been the subject of many reports. Most of the discussion has been related to the dermatitis and sensitization observed during curing and, as Bourne, *et al.*,[1] state, the cause of most of this appears to be the uncombined amine catalyst which is present in the epoxy resin/amine mixture. Lee, *et al.*,[2] tested irritation and sensitization from an uncured resin, an uncured resin modifier and four amine curing agents, utilizing human patch tests, as did Key, *et al.*,[3] with several epoxy resin components. In some instances the uncured resins and modifiers were found to be irritating and sensitizing. Dorman[4] also reported severe skin disorders from handling liquid epoxy resins and amine-type curing agents. Hine, *et al.*,[5] and Lee and Neville[6] emphasize that if there is no skin contact, there will be no dermatitis; that good personal hygiene is all that is required to prevent injuries.

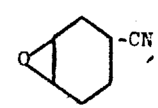
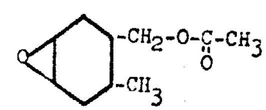
There are available only a few publications on the toxicity and carcinogenicity of the epoxy monomers. In the area of toxicity these include the reports of Hine, *et al.*,[7] who studied six epoxy resins, and of Cornish and Block[8] who reported on the range-finding and/or inhalation toxicity of one uncured resin, two resin modifiers and six amine curing agents. White and Shaeffer[9] presented physiological irritation, sensitization, and toxicity data upon several resins and monomers. The most complete collection of health information on the epoxy compounds is the chapter on this subject by Hine and Rowe in the book edited by Patty.[10]

Reference has been made by Walpole and Williams[11] to the possibility of qualitative changes produced in particular blood components. Kodama, *et al.*,[12] reported that less complicated polyfunctional epoxides and monofunctional epoxides with auxiliary active groups, parentally administered, resulted in a decrease in the number of nucleated cells in the bone marrow, in the total number of circulating white cells, and in the predominant white cell type in the peripheral blood.

Hendry, *et al.*,[13] cited the tumor-inhibitory and cytotoxic activity of many mono- and di-(bis)epoxides. They concluded that marked activity in these tests had been shown only by certain members of the bisepoxide class; in particular butadiene dioxide, vinyl cyclohexene dioxide, certain N:N-diglycidyl derivatives of primary aromatic amines and diglycidyl ethers of aliphatic diols. The monoepoxides were inactive. Intraperitoneal injec-

Table I
Structure and Identification of Tested Epoxides

Number	Material	Formula
1	Ethylene oxide	$\underset{O}{CH_2-CH_2}$
2	Propylene oxide	$\underset{O}{CH_2-CH}-CH_3$
3	1,2-Epoxybutane	$\underset{O}{CH_2-CH}-CH-CH_3$
4	Epichlorhydrin	$\underset{O}{CH_2-CH}-CH_2-Cl$
5	N-Glycidyldiethylamine	$\underset{O}{CH_2-CH}-CH_2-N(CH_2-CH_3)_2$
6	Butyl glycidyl ether	$\underset{O}{CH_2-CH}-CH_2-O-C_4H_9$
7	Epoxidized soybean oil	Indefinite
8	Butadiene monoxide	$\underset{O}{CH_2-CH}-CH=CH_2$
9	Glycidyl acrylate	$\underset{O}{CH_2-CH}-CH_2-O-\underset{O}{C}-CH=CH_2$
10	Glycidyl oleate ✓	$\underset{O}{CH_2-CH}-CH_2-O-\underset{O}{C}-(CH_2)_7-CH=CH-(CH_2)_7-CH_3$
11	Styrene oxide	
12	Glycidyl benzoate	
13	Phenyl glycidyl ether	

14	2,3-Epoxy-2-methylpropyl acrylate	$\underbrace{CH_2-\overset{CH_3}{C}}_{O}-CH_2-O-\underset{O}{C}-CH=CH_2$
15	1,4-Dichloro-2,3-epoxybutane	$Cl-CH_2-\underbrace{CH-CH}_{O}-CH_2-Cl$
16	Diisobutylene oxide	Mixture of $CH_3-\overset{CH_3}{\underset{CH_3}{C}}-\underbrace{\overset{H}{C}-\overset{CH_3}{C}}_{O}-CH_3$ and $CH_3-\overset{CH_3}{\underset{CH_3}{C}}-CH_2-\underbrace{\overset{CH_3}{C}-CH_2}_{O}$
17	Ethyl 2,3-Epoxybutyrate	$C_2H_5-O-\underset{O}{C}-\underbrace{CH-CH}_{O}-CH_3$
18	2,3-Epoxy-2-ethylhexanol	$CH_3-CH-CH_2-\underbrace{CH-\overset{C_2H_5}{C}}_{O}-CH_2OH$
19	Triisobutylene oxide	Mixture of Isomers of Empirical formula $C_{12}H_{24}O$
20	2-Ethylhexyl 9,10-epoxystearate	$C_4H_9-\overset{C_2H_5}{\underset{H}{C}}-CH_2-O-\underset{O}{C}-(CH_2)_7-\underbrace{CH-CH}_{O}-(CH_2)_7-CH_3$
21	Allyl 9,10-Epoxystearate	$CH_2=CH-CH_2-O-\underset{O}{C}-(CH_2)_7-\underbrace{CH-CH}_{O}-(CH_2)_7-CH_3$
22	3,4-Epoxycyclohexanecarbonitrile	
23	3,4-Epoxy-6-methylcyclohexylmethyl acetate	
24	Ethyl 3-Oxatricyclo-[3.2.1.0^{2,4}]octane-6-carboxylate	

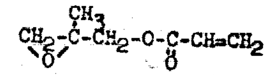
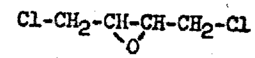
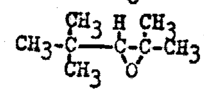
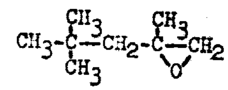
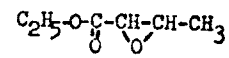
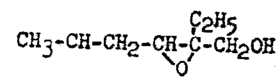
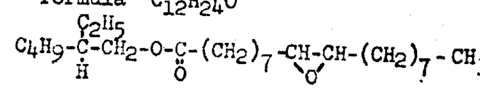
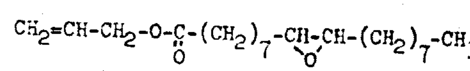
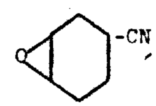
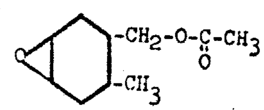
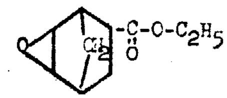
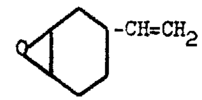
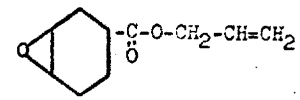
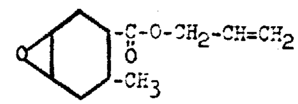
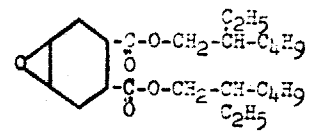
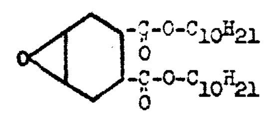
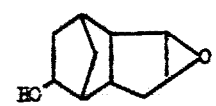
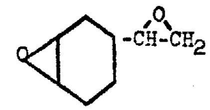
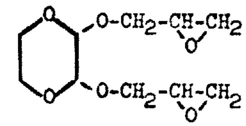
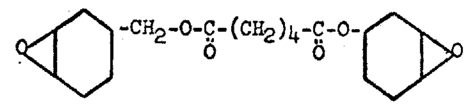
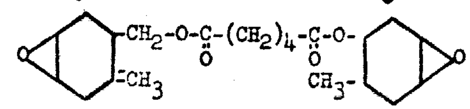
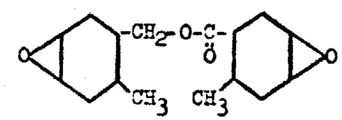
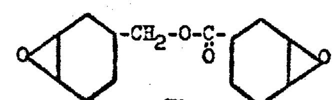
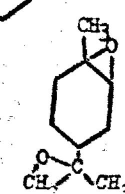
14	2,3-Epoxy-2-methylpropyl acrylate	
15	1,4-Dichloro-2,3-epoxybutane	
16	Diisobutylene oxide	Mixture of  and 
17	Ethyl 2,3-Epoxybutyrate	
18	2,3-Epoxy-2-ethylhexanol	
19	Triisobutylene oxide	Mixture of Isomers of Empirical formula $C_{12}H_{24}O$
20	2-Ethylhexyl 9,10-epoxystearate	
21	Allyl 9,10-Epoxystearate	
22	3,4-Epoxycyclohexanecarbonitrile	
23	3,4-Epoxy-6-methylcyclohexylmethyl acetate	
24	Ethyl 3-Oxatricyclo-[3.2.1.0^{2,4}]octane-6-carboxylate	

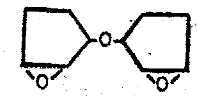
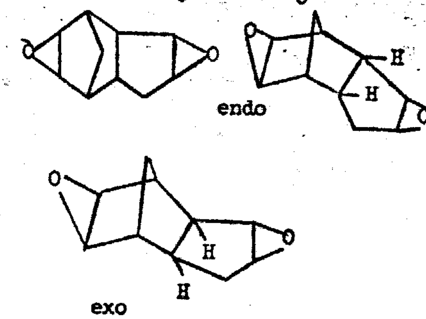
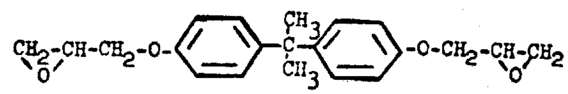
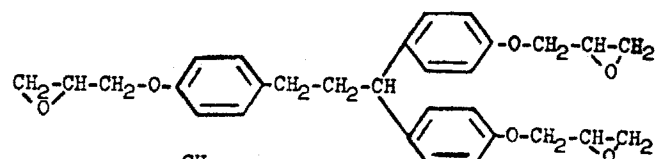
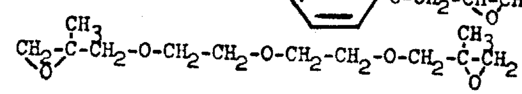
TABLE I *(continued)*

Number	Material	Formula
		
25	Vinylcyclohexene monoxide	
26	Allyl 3,4-Epoxycyclohexanecarboxylate	
27	Allyl 3,4-Epoxy-6-methylcyclohexanecarboxylate	
28	Di(2-Ethylhexyl) 4,5-epoxycyclohexane-1,2-dicarboxylate	
29	Di(Isodecyl) 4,5-Epoxycyclohexane-1,2-dicarboxylate	
30	4-Oxatetracyclo-/6.2.1.0^{2,7}0^{3,5}/-undecan-9-ol	
31	Epoxidized 2-Ethylhexyl ester of tall oil fatty acid	Indefinite
32	Alkyl epoxytallate	Indefinite
33	Epoxidized CARBOWAX [illegible] ester of tall oil fatty acid	Indefinite

34	Epoxidized Ethylene glycol ester of tall oil fatty acid	Indefinite
35	Calcium salt of epoxidized tall oil fatty acid	Indefinite
36	Magnesium salt of epoxidized tall oil fatty acid	Indefinite
37	Cadmium 9,10-epoxystearate	$Cd[O\text{-}\underset{O}{C}\text{-}(CH_2)_7\text{-}\underset{O}{CH\text{-}CH}\text{-}(CH_2)_7\text{-}CH_3]_2$
38	Calcium 9,10-epoxystearate	$Ca[O\text{-}\underset{O}{C}\text{-}(CH_2)_7\text{-}\underset{O}{CH\text{-}CH}\text{-}(CH_2)_7\text{-}CH_3]_2$
39	Magnesium 9,10-epoxystearate	$Mg[O\text{-}\underset{O}{C}\text{-}(CH_2)_7\text{-}\underset{O}{CH\text{-}CH}\text{-}(CH_2)_7\text{-}CH_3]_2$
40	Calcium 2,3-Epoxy-2-ethylhexanoate	$Ca(O\text{-}\underset{O}{C}\text{-}\underset{O}{\overset{C_2H_5}{C}\text{-}CH}\text{-}CH_2\text{-}CH_2\text{-}CH_3)_2$
41	Tetrapropylene oxide	Indefinite
42	Butadiene dioxide	$\underset{O}{CH_2\text{-}CH}\text{-}\underset{O}{CH\text{-}CH_2}$
43	1,2-7,8-Diepoxyoctane	$\underset{O}{CH_2\text{-}CH}\text{-}(CH_2)_4\text{-}\underset{O}{CH\text{-}CH_2}$
44	Bis-(2,3-epoxy-2-methylpropyl)ether	$\underset{O}{CH_2\text{-}\overset{CH_3}{C}}\text{-}CH_2\text{-}O\text{-}CH_2\text{-}\underset{O}{\overset{CH_3}{C}\text{-}CH_2}$
45	Bis-(3,4-epoxybutyl)ether	$\underset{O}{CH_2\text{-}CH}\text{-}CH_2\text{-}CH_2\text{-}O\text{-}CH_2\text{-}CH_2\text{-}\underset{O}{CH\text{-}CH_2}$
46	Ethylene glycol bis-(2,3-epoxy-2-methylpropyl)ether	$\underset{O}{CH_2\text{-}\overset{CH_3}{C}}\text{-}CH_2\text{-}O\text{-}CH_2\text{-}CH_2\text{-}O\text{-}CH_2\text{-}\underset{O}{\overset{CH_3}{C}\text{-}CH_2}$
47	2,3-Epoxy-2-ethylhexyl 9,10-epoxystearate	$CH_3\text{-}CH_2\text{-}CH_2\text{-}\underset{O}{CH\text{-}\overset{C_2H_5}{C}}\text{-}CH_2\text{-}O\text{-}\underset{O}{C}\text{-}(CH_2)_7\text{-}\underset{O}{CH\text{-}CH}\text{-}(CH_2)_7\text{-}CH_3$
48	2-Ethyl-1,3-hexanediol bis(9,10-epoxystearate)	$C_3H_7\text{-}CH\text{-}\overset{C_2H_5}{CH}\text{-}CH_2\text{-}O\text{-}\underset{O}{C}\text{-}(CH_2)_7\text{-}\underset{O}{CH\text{-}CH}\text{-}(CH_2)_7\text{-}CH_3$ $O\text{-}\underset{O}{C}\text{-}(CH_2)_7\text{-}\underset{O}{CH\text{-}CH}\text{-}(CH_2)_7\text{-}CH_3$

TABLE I *(concluded)*

Number	Material	Formula
49	Glycidyl sorbate (dimer)	Indefinite
50	Vinylcyclohexene dioxide	
51	2,3-Bis(glycidyloxy)-1,4-dioxane	
52	Bis-(3,4-epoxycyclohexylmethyl) adipate	
53	Bis-(3,4-epoxy-6-methylcyclohexylmethyl) adipate	
54	3,4-Epoxy-6-methylcyclohexylmethyl 3,4-epoxy-6-methylcyclohexanecarboxylate	
55	3,4-Epoxycyclohexylmethyl 3,4-epoxycyclohexanecarboxylate	
56	Limonene dioxide	
57	Bis-(2,3-Epoxycyclopentyl) ether	

58	endo-Dicyclopentadiene dioxide	
59	Dicyclopentadiene dioxide, 70% exo isomers, 30% endo isomers	
60	3,4-Epoxy-6-methylcyclohexylmethyl ester of tall oil fatty acid	Indefinite
61	2,2,-Bis-[p-(2,3-glycidyloxy)phenyl]propane	
62	1,1,3-Tris[p-(glycidyloxy)phenyl]propane	
63	Bis-(2-(2',3'-Epoxy-2'-methylpropoxy)-ethyl) ether	
64	Modified bis-phenol diglycidyl ethers	Indefinite
65	Modified bis-phenol diglycidyl ethers	Indefinite
66	Bis-(2,3-epoxy-2-methylpropyl) ether of CARBOWAX 200	Indefinite

tion of vinyl cyclohexene dioxide produced one sarcoma, while mouse skin painting resulted in a high yield of tumors. They concluded that evidence for skin carcinogenicity was not so strong for butadiene dioxide, although that compound produced mutations in a strain of Penicillium.

McCammon, *et al.*,[14] stated that three diepoxides produced mouse skin cancer; and that all 20 compounds tested suppressed sebaceous glands and caused epithelial changes, frequently marked by hyperplasia. Hine, *et al.*,[5] reported on the absence of carcinogenic action of a 5% acetone solution of a liquid bisphenol-based epoxy resin, and on the low, but positive, carcinogenic action of a liquid aliphatic epoxy resin painted three times weekly at this 5% level. The four tumors reported by Hine appeared after 10, 16, 22, and 23 months of painting.

It is worthy of note that the highly positive action of the first vinyl cyclohexene dioxide paintings by Hendry *et al.*,[13] were made "with one drop (ca. 16 mg) of the compound, five times weekly for a total of 12 months. Skin papillomata began to appear within a few months, and many of these subsequently became malignant." In this same 1951 article they stated that in a further experiment a highly purified sample, more toxic and more active cytotoxically, was being applied as "a solution in acetone (three parts)" to the skin of mice at the rate of one drop per animal twice weekly. "The first papilloma made its appearance at two months."

However, in their 1954 article, these authors[15] stated that "we have already reported the production of carcinoma of the skin in mice by painting with vinylcyclohexene dioxide in acetone" (and they here refer to the page in their 1951 article quoted above) "but, repetition of this experiment with a more highly purified sample of this diepoxide has failed to yield tumors."

These references are confusing to analyze. No statement was made in the 1951 article of whether the first study was run on an undiluted or diluted sample. The compound was painted five times a week. Then they stated, painting twice a week with a 25% solution in acetone of a different purified sample resulted in the first papilloma in two months. Still, in 1954, they state that the purified sample (presumed to be the same one referred to in their first article) was not productive of tumors. They presume that the difference in action between the samples was due to the difference in purity, but it could equally as well have been produced by differences in concentration or frequency of painting.

Some of the following range-finding toxicity data have been reported in previous papers[16] from this laboratory, with the latest issued in 1962.[17] However, they are resummarized here together with previously unpublished data to assist in the attempt to portray the over-all picture of toxicity of these epoxides. Furthermore, the experimental cancer studies on many mono- and diepoxides have not been published previously. It is important to ascertain whether one can justifiably make a general statement, such as is currently prevalent, that diepoxides are carcinogenic. White[18] spoke on the subject of considering the members of this chemical family as toxicological individuals. It is believed that the examination of the toxicological and carcinogenic nature of a large number of these family members will elucidate the soundness of this statement.

Methods

The toxicological methods currently used in this laboratory were discussed in our latest communication[17] on the range-finding test and will, therefore, not be repeated here. Sensitization of guinea pigs was determined by a technique consisting of eight intracutaneous injections (three per week on alternate days) of 0.1 ml of the diluted epoxy materials. A three-week incubation period was followed by a challenge dose, and examinations for possible sensitization reactions were made 24 and 48 hours thereafter.

The lifetime tumorigenicity procedure used was similar to that reported by Weil and Condra.[19] Basically, the hair was removed with electric clippers, as necessary, from the backs of 90-day old mice. The mice received three applications per week—one brushful of chemical being applied to the midline of the back on Monday, Wednesday, and Friday. Observations were made for papillomas and carcinomas during each painting period. Groups of 30 to 40 mice were used for each material tested.

Mice of the C3H strain were used in the majority of the studies. All liquid materials were applied undiluted if not too toxic; in solution in a solvent if a solid or if unusually toxic. The mice were observed until death; quite important in the assay of tumorigenic potential of materials such as epoxides which often result in tumors late in the life-span of the mouse, *if at all.* Several, nonstandard experiments were made with the compound numbered 54. These will be discussed later.

Whenever a tumor was seen on the skin, a plot of its position was made and a record of its progress was kept. The median tumor or cancer latent periods were calculated by the method suggested by Horton.[20] These latent periods are the lengths of time necessary to reach a 50% tumor or cancer index. The indices are 100 times the number of mice with skin tumors or with cancers, divided by the "effective group" where "effective group" is the number of mice given adequate exposure. This is the original number of mice employed less the number that have died without tumors. It is, therefore, a variable number that decreases by one with each nontumor death. It is, however, arbitrarily held constant after the time of the appearance of a tumor in the median tumor-bearing mouse.

The sebaceous gland test, somewhat similar to that described by Suntzeff, *et al.*,[21] as used in this laboratory involved the daily application of one brushstroke of a chemical under test; five days the first week and four the second. The day after the last painting all surviving mice were killed; the skin subsequently was fixed, embedded, sectioned and stained. The number of sebaceous glands per square centimeter of skin was counted and related to the number present in the skin of control mice. We arbitrarily use the following grading system:

Grade	*Percentage of Control Mean*
0	76 to 100
1	51 to 75
2	26 to 50
3	0 to 25

An advantage of this nine-application test other than its reduction in elapsed time, is its indication of the toxicity of the chemical before it is used for lifetime painting. If the mice died in this two-week period (labelled T for toxic in Table III) lower concentrations of the chemical were used in lifetime tests.

Results and Discussion

The results of the range-finding toxicity tests, the sebaceous gland suppression and the lifetime skin-painting tests are summarized in Tables II and III. The materials are chemically identified, whenever possible, in Table I. Herein they are numbered and these numbers are used for reference in the subsequent tables. All tests have not been performed on each material. Furthermore, this compendium of epoxide toxicity and carcinogenicity data, although relatively large in relation to data already published, is still represented by only a few members of certain structural subdivisions. Nevertheless, the structurally similar members have been placed as near each other in the tables as possible, with the following exceptions: 13 of the 60 materials for which data are incomplete are presented at the end of Table II. Similarly, in Table III, when only the sebaceous gland test was run, the entries are again made at the end of the table. The terminally located monoepoxides are numbered one through 14; those with the one epoxy group located internally are numbered 15 through 36. The diepoxides tested plus a few materials with more than two epoxy groups are numbered 37 through 66. Furthermore, each table, as far as possible, follows this order: saturated aliphatics, unsaturated aliphatics, alicyclics and aromatics. This arrangement was used to reveal relationships between structure and toxicity, if any exist.

Toxicity

A wide range of toxicity and irritation of epoxy monomers is revealed by the data in Table II. No consistent correlations with structure are evident. Accordingly, each epoxide considered for utilization should be studied as an entirely new substance, rather than relying upon data already obtained on similar structures.

TABLE II

Experimental Toxicity Results of Tested Epoxides

Number	Single Oral LD_{50} for Rats Ml./Kg.	Single Skin Penetration LD_{50} for Rabbits Ml./Kg.	Concentrated Vapor Inhalation by Rats Maximum for No Death	Inhalation of Metered Vapor Concentration by Rats: Concentration, PPM.	Time, Hour	Mortality	Irritation on Uncovered Rabbit Belly	Corneal Injury in Rabbits	Sensitization of Guinea Pigs No. Sensit. / No. Tested
1	0.33(0.29-0.36)*	-	-	8,000 4,000	4 4	6/6 0/6	-	-	-
2	0.63(0.54-0.73)	1.50(1.07-2.10)	5 min.§	4,000	4	4/6	1	5	-
3	1.41(0.88-2.29)	2.10(1.50-2.95)	5 min.§	8,000 4,000	4 4	6/6 1/6	1	4	-
4	0.21(0.20-0.23)*	1.3	-	250	8	4/6	2	4	0/18
5	0.42(0.26-0.68)	0.79(0.59-1.07)	30 min.	2,000 1,000	4 4	6/6 0/6	6	9	-
6	2.05(1.56-2.69)*	2.52(1.28-4.97)	2 hours	4,000	4	1/6	1	5	16/17
7	22.5(14.0-36.0)	> 20	8 hours	-	-	-	2	1	0/20
9	0.21(0.15-0.30)*	0.40(0.18-0.92)	30 min.	125 62.5	4 4	6/6 0/6	7	10	-
10	3.52(3.35-3.69)*	8	8 hours	-	-	-	2	1	2/3
11	2.83	0.89(0.49-1.63)	4 hours	1,000	4	2/6	5	2	6/11
13	4.26(3.90-4.66)*	1.50(1.07-2.10)	8 hours	-	-	-	5	2	1/18
15	0.71 *	2.83	2 hours	-	-	-	2	2	-

Number									
16	4.92(3.75-6.46)*	14.1(8.74-22.9)	10 min.	4,000	4	2/6	3	2	0/10
17	0.50(0.32-0.79)	2.83	8 hours	-	-	-	1	2	-
18	5.05(4.49-5.68)	3.15(2.33-4.26)	8 hours	-	-	-	1	5	-
19	6.69(4.11-10.90)	14.1	8 hours	-	-	-	6	1	0/20
20	30.8(24.9-38.1)	> 20	8 hours	-	-	-	3	1	0/20
21	1.41(0.88-2.29)	15.9(11.7-21.5)	8 hours	-	-	-	3	1	-
22	1.23(0.94-1.62)	0.99(0.73-1.34)	8 hours	-	-	-	2	5	-
23	9.8(6.1-12.0)	7.94(5.87-10.73)	8 hours	-	-	-	2	2	-
24	4.76(3.28-6.75)	3.54	8 hours	-	-	-	2	2	-
25	2.00(1.28-3.13)	2.83	2 hours	-	-	-	2	5	0/19
27	0.50(0.32-0.79)	2.83(0.93-8.58)	8 hours	-	-	-	3	1	-
28	> 64	> 20	8 hours	-	-	-	2	1	-
29	> 64	> 20	8 hours	-	-	-	2	2	-
30	3.13(2.68-5.21)	> 10.0	8 hours	-	-	-	2	7	-
31	22.6	> 20	8 hours	-	-	-	3	1	-
32	45.3(32.8-62.3)	15.9(11.7-21.5)	8 hours	-	-	-	2	1	-

TABLE II (continued)

Number	Single Oral LD_{50} for Rats Ml./Kg.	Single Skin Penetration LD_{50} for Rabbits Ml./Kg.	Concentrated Vapor Inhalation by Rats Maximum for No Death	Inhalation of Metered Vapor Concentration by Rats: Concentration, PPM.	Time, Hour	Mortality	Irritation on Uncovered Rabbit Belly	Corneal Injury in Rabbits	Sensitization of Guinea Pigs No. Sensit./No. Tested
33	39.4(30.0-51.7)	> 20	8 hours	-	-	-	2	1	-
42	0.088 *	0.035	< 15 min.	125	4	1/5	6	10	18/18
43	1.07(0.77-1.50)	0.32(0.23-0.43)	4 hours	-	-	-	6	7	-
44	1.68(1.03-2.74)	1.25(0.84-1.85)	8 hours	-	-	-	4	7	4/19
45	1.07(0.77-1.50)	0.25(0.17-0.37)	8 hours	-	-	-	5	7	-
46	7.46(5.35-10.41)	3.15(2.33-4.26)	-	-	-	-	2	5	0/17
50	2.83 *	0.62(0.25-1.57)	8 hours	-	-	-	5	7	0/16
51	1.07(0.77-1.50) *	1.59(1.17-2.20)	8 hours	-	-	-	4	7	-
52	4.29(3.07-5.98)	> 20	8 hours	-	-	-	2	1	-
53	5.19(4.20-6.41)	> 10	8 hours	-	-	-	2	1	0/12
54	4.92(3.75-6.46)	> 10	8 hours	-	-	-	2	1	0/15
55	4.49(1.81-11.2)	> 20	8 hours	-	-	-	2	1	-
56	5.63 *	1.77	8 hours	-	-	-	4	3	0/17
57	2.14(1.54-2.99)	> 5.0	8 hours	-	-	-	1	3	0/13
58	0.21(0.13-0.34) *	< 8.0 *	8 hours	-	-	-	-	-	1/14
59	0.31(0.25-0.38) *	3.18(2.35-4.29) *	-	-	-	-	-	-	-

Number									
60	26.0(16.9-39.9)	> 20	8 hours	-	-	-	2	1	-
61	19.6(14.9-25.7)	> 20	-	-	-	-	1	1	19/20
64	-	-	8 hours	-	-	-	1	1	20/20
12	1.41 *	-	-	-	-	-	-	1	-
34	53.8(28.3-103)	-	-	-	-	-	-	-	-
35	> 20 *	-	-	-	-	-	-	-	-
36	> 10 *	-	-	-	-	-	-	-	-
37	> 16 *	-	-	-	-	-	-	-	-
38	> 40 *	-	-	-	-	-	-	-	-
39	33.2(23.0-42.9)*	-	-	-	-	-	-	-	-
40	> 20 *	-	-	-	-	-	-	-	-
41	1.8 *	-	-	-	-	-	-	-	-
47	45.2	-	-	-	-	-	3	4	-
48	> 64	-	-	-	-	-	-	-	-
49	5.74(5.28-6.25)*	-	-	-	-	-	2	2	-
62	-	> 8 *	-	-	-	-	-	-	14/16

* as gm./kg. in a suitable vehicle.
§ inhalation time shown killed all six rats.

Table III

Experimental Carcinogenicity Results of Tested Epoxides

	Results of Life-Span Cancer Studies														Sebaceous Gland Test		
		No. of Mice Alive at Month			Appearance of First Tumor		Total No. of Mice With					Median Latent Period (Months)				
Number	Concentration	12	17	24	Months of Painting	No. of Mice Alive	Tumors	Cancer	Maximum No. of Months Painted	Tumor Index	Cancer Index	Tumor	Cancer	Concentration	% of Control Mean	Grade
4	U	37	30	1	-	-	0	0	25	0.0	0.0	-	-	-	-	-
7	U	36	26	0	-	-	0	0	24	0.0	0.0	-	-	U	100	0
9	10-A	32	19	5	-	-	0	0	> 26	0.0	0.0	-	-	-	-	-
11	10-A	18	2	0	-	-	0	0	18	(0.0)	(0.0)	-	-	U	21.7*	3
	5-A	37	33	17	-	-	0	0	> 24	0.0	0.0	-	-	10-A	37.0*	2
14	50-A	12	4	0	-	-	0	0	20	(0.0)	(0.0)	-	-	-	-	-
	10-A	35	33	17	-	-	0	0	> 26	0.0	0.0	-	-	-	-	-
16	U	37	25	0	-	-	0	0	24	0.0	0.0	-	-	U	52.9	1
19	10-A	33	17	0	-	-	0	0	21	0.0	0.0	-	-	U	27.0*	2
														10-A	70.1	1
25	U	25	17	0	10	29	1	0	22	3.4	0.0	-	-	U	82.0	0
29	U	33	29	0	-	-	0	0	24	0.0	0.0	-	-	U	100	0
30	U	32	29	6	-	-	0	0	26	0.0	0.0	-	-	-	-	-
31	U	30	23	6	-	-	0	0	27	0.0	0.0	-	-	-	-	-
42	10-A	25	4	0	18	3	2	1	20	66.7	33.3	18.5	∞	U	-	T
														10-A	40.9*	2

Number																
44	20-A	6	2	0	4	27	7	0	19	38.9	0.0	> 19	-	-	-	-
46	50-A	37	32	7	-	-	0	0	26	0.0	0.0	-	-	-	-	-
50	10-A	18	6	0	17	6	3	1	21	75.0	25.0	19.5	∞	U 10-A	- 100	T 0
53	U	35	25	2	-	-	0	0	25	0.0	0.0	-	-	-	-	-
54							(SEE TABLE IV)							U	4.5*	3
56	U	33	30	8	-	-	0	0	28	0.0	0.0	-	-	-	-	-
57	30-A	18	10	0	-	-	0	0	21	0.0	0.0	-	-	U 10-A	- 100	T 0
58	25-DMP	35	24	5	-	-	0	0	28	0.0	0.0	-	-	30-A	93.7	0
59	40-DMP	34	30	14	-	-	0	0	> 26	0.0	0.0	-	-	-	-	-
60	U	36	30	3	-	-	0	0	28	0.0	0.0	-	-	U	100	0
61	U U	26 36	14 26	1 0	- 16	- 32	0 1	0 0	27 23	0.0 3.1	0.0 0.0	- > 24	- -	- -	- -	- -
62	50-A	32	22	0	-	-	0	0	23	0.0	0.0	-	-	-	-	-
63	U	38	31	9	-	-	0	0	27	0.0	0.0	-	-	-	-	-
64	U	32	22	1	-	-	0	0	25	0.0	0.0	-	-	-	-	-
65	U U	34 18	28 11	4 1	23 9	4 24	3 3	3 0	26 25	75.0 12.5	75.0 0.0	23 > 24	24 -	- -	- -	- -
66	U	39	31	9	-	-	0	0	28	0.0	0.0	-	-	-	-	-

TABLE III *(continued)*

Materials Tested on Sebaceous Gland Test Only—Not on Life-Span Cancer Studies

Number	Sebaceous Gland Test: Concentration	% of Control Mean	Grade
8	U	100	0
15	U	100	0
17	U	100	0
18	U	100	0
20	U	100	0
21	U	11.9*	3
26	U	-	T
	10-A	1.7*	3
27	U	-	T
	10-A	63.0	1
28	U	100	0
39	10-CO	100	0
43x	5-A	29.2*	2
51x	U	46.6*	2
	25-A	64.6	1
52x	U	83.1	0
55	U	99.3	0

Abbreviations: Concentration U = undiluted T = Toxic
A = acetone; e.g. 10-A = 10% solution in acetone
CO = corn oil; e.g. 10-CO = 10% solution in corn oil
DMP = dimethyl phthalate; e.g. 25-DMP = 25% solution in dimethyl phthalate

* Significantly lower than control mean
x Currently being tested in life-span studies

Carcinogenicity

Twenty-eight of the epoxides were tested by lifetime mouse skinpainting for cancer potential (Table III). Two of these proved to be too toxic in our first tests to determine their true carcinogenicity. These were styrene oxide and 2,3-epoxy-2-methylpropyl acrylate (numbers 11 and 14) originally tested as 10% and as 50% dilutions in acetone. As only two and four mice were alive after 17 months of painting, they were retested at lower concentrations.

All of the other 26 tests were successfully completed. In addition to the two previously mentioned monoepoxides, nine others have been studied, seven of which were applied undiluted. None of these eleven were tumorigenic to the mouse skin. Seventeen diepoxides have been successfully tested; in other words an adequate number of mice survived until tumors developed or until at least 17 months of painting were completed. Five of these 17 resulted in some tumorigenicity, four of the five had tumor indices above 50%. These four include:

	Material Number
butadiene dioxide	42
vinylcyclohexene dioxide	50
3,4-epoxy-6-methylcyclohexylmethyl 3,4-epoxy-6-methyl cyclohexane carboxylate	54
modified bis phenol diglycidyl ether	65

The other diepoxide that resulted in some tumors, but with tumor index less than 50% was bis-(2,3-epoxy-2-methylpropyl) ether—material number 44. Material number 61 resulted in only one papilloma when tested once; none in a retest.

The diepoxide numbered 54, identified above, has been tested in five lifetime skin-painting studies. These are summarized in Table IV. In all five the tumor indices were 60% or higher for the undiluted material; with cancer indices of 14% to 67%. Two of the highest tumor and cancer indices were obtained using the especially purified D-1 sample (tumor indices of 100 and 81% with cancer indices of 67 and 58%). The former was with C57-leaden mice; all of the others with C3H with the exception of those on study 23 where mice one year of age at first painting were used. These were of the 1C3F1 and C31F1 strains. The question to be answered by this study was, if the mice were older at the start of the study, would they develop tumors earlier than the younger mice normally used?

The median latent period (M.L.P.) is a measure of the time when the median tumor-bearing mouse develops a tumor. For the positive control material, a 0.2% solution of methyl cholanthrene in acetone, this M.L.P. ranged from 3 to 5 months. For the four diepoxides, for which this could be measured, other than number 54, the M.L.P. was 18.5, >19.5 and 23 or >24 months of painting (Table III). For number 54 in the other studies on the undiluted material these M.L.P.s were 15.2, 15.4, 17.5, 18.3 and 19 months of painting. The two groups of mice, one year of age at first painting, still required 14.7 or 15.3 months of painting to reach the M.L.P. Therefore, no marked reduction in this latent period resulted from using older mice at first painting.

In study 22, an attempt was made to determine whether the addition of dodecane, reported by Horton[22] to be a tumor accelerator, would shorten the latent period. Instead, a 50:50 mixture of dodecane and the diepoxide resulted in the longest latent period, namely, 22.4 months. It is notable that 5% acetone solutions of this diepoxide were nontumorigenic. This is similar to the concentration used by Hine, *et al.*,[4] for their work with two epoxides. Whenever toxicity was sufficiently low, the epoxides were painted undiluted. Dilution will abort or delay tumor production by materials which are carcinogenic when painted undiluted. This has been reported previously.[19,23,24,25] However, if undiluted materials are to be handled by workmen, the only proper test of hazard is to apply these materials experimentally in as concentrated a manner as is practical. Nine of the 17 diepoxides were tested undiluted while the others were tested as 10 to 20% solutions in acetone or in dimethyl phthalate. Some of these latter had been shown to be toxic when applied undiluted, either in the sebaceous gland test or in previous long-term tests in this laboratory. Wherever possible, the concentrations reported in the tables are for studies where enough mice survived a sufficient length of time to allow a conclusion to be drawn.

It is apparent that the presence of two epoxy groups in a molecule is not always productive of tumors. While none of the eleven monoepoxides so far tested were tumorigenic, only five of the 17 compounds with two or more epoxy groups, were tumorigenic. As was the case in the possible relationship of structure and toxicity, only general statements can be made of the structure *versus* cancer relationship. Perhaps it is necessary for a material to have two epoxy rings to be tumorigenic, but not all of the diepoxides are tumorigenic. Included with the diepoxides is one material containing more than two epoxy radicals. This is 1,1,3-tris [p-(2,3-glycidyloxy) phenyl] propane (number 62) and, as a 50% solution in acetone, it was not tumorigenic.

Sebaceous Gland Suppression

The sebaceous gland suppression for certain epoxides is summarized in Table III. Arbitrarily we have graded the relationship between the mean number of sebaceous glands per square centimeter of skin of epoxide-dosed mice to that of the control mice. If the material was applied undiluted, it was compared to a distilled water control group. If applied in acetone, results with this solvent were used for the statistical and grading comparison. The asterisks on the means in the tables indicate that, on the raw data count basis, the means of the epoxide-treated group were statistically different from those of their controls.

Twelve substances were tested by both the sebaceous gland (S.G.T.) and the lifetime tests (L.T.T.). In seven of the nine cases when the L.T.T. was negative (3.4 in one; 0.0 on the other eight) the S.G.T. grade was '0' or '1.' One of these nine was graded '2' and another graded '3,' was toxic as a 10% solution in acetone in the L.T.T. Probably of more importance is the relationship in the three lifetime tests where the epoxide skin applications resulted in tumor indices of greater than 50%. Two of the three resulted in significantly depressed numbers of sebaceous glands, *i.e.*, grades of '2' or '3.' In the S.G.T. the other long-term carcinogen was toxic undiluted and grade '0' as a 10% solution in acetone.

There were four instances where statistically significant sebaceous gland suppression resulted and where lifetime tests have been run. Triisobutylene oxide (number 19) was positive undiluted and negative as a 10% solution in acetone in the S.G.T., while negative in the L.T.T., using a 10% solution in acetone. Styrene oxide (number 11) was significant in sebaceous gland suppression both undiluted and at a 10% dilution in acetone. The latter concentration was toxic on long-term application; a 5% solution in acetone was not tumorigenic in the L.T.T. Butadiene dioxide, (number 42), at 5% and at 10% concentrations in acetone significantly suppressed subaceous glands, and, furthermore, a tumor index of 67% resulted with a 10% solution in acetone in the L.T.T. Similarly, 3,4-epoxy-6-methyl cyclohexylmethyl 3,4-epoxy-6-methyl cyclohexane carboxylate (number 54) definitely suppressed sebaceous glands and has been repeatedly shown to be a mouse-skin carcinogen. Vinyl cyclohexene dioxide, (number 50), a lifetime test carcinogen as 10% concentration in acetone, was toxic undiluted and negative at 10% in acetone in the S.G.T.

Thus, it is apparent, even though the number of comparisons possible is few, the sebaceous gland test resulted in either toxicity or suppression whenever the long-term test was positive. False-positive sebaceous gland results occurred with triisobutylene oxide and styrene oxide. If the former had been tested in the L.T.T. at a high concentration it might have resulted in tumors. The latter was so toxic in the L.T.T. that comparisons are difficult.

Several sebaceous gland tests have been run unaccompanied by long-term tests. The following is a summary of S.G. grade *versus* epoxide type for these, as well as for those where both tests have been run:

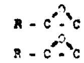
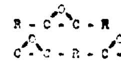
	Sebaceous Gland Grade '0' or '1'	'2' or '3'	*Per Cent of Total in Grade '2' or '3'*
	2	1	33
	9	3	25
	7	4	36

TABLE IV
Carcinogenicity of Material Number 54

Study	Strain	Concentration	No. of Mice Alive at 1 Year	No. of Mice Alive at 17 Months	No. of Mice Alive at 24 Months	Appearance of First Tumor: Months of Painting	Appearance of First Tumor: No. of Mice Alive	Total No. of Mice With Tumors	Total No. of Mice With Cancers	Maximum No. of Months Painted	Tumor Index	Cancer Index	Median Latent Period (M.L.D.) Months: Tumor	Median Latent Period (M.L.D.) Months: Cancer
13	C3H	U	20	9	0	15	15	9	3	21	60.0	30.0	17.5	> 24
16	C57L	U (D-1)	11	1	0	15	3	3	2	18	100.0	66.7	15.4	16.5
17	C3H	U	38	23	0	10	39	20	9	24	76.9	34.6	18.3	> 24
		5%-A	38	27	0	-	-	0	0	24	0.0	0.0	-	-
		U (D-1)	38	25	0	13	37	29	21	23	80.6	58.3	15.2	19.3
		5%-A	38	28	0	-	-	0	0	24	0.0	0.0	-	-
22	C3H	U	31	17	0	15	21	14	7	23	77.8	38.9	19.0	> 24
		50 parts + 50 parts dodecane	37	23	6	11	37	9	6	25	60.0	40.0	22.4	> 24
23	1C3F1*	U	18/21	8	0	13	18	13	6	20	72.2	33.3	15.3	19.6
	C31F1*	U	12/23	1	0	13	9	7	1	18	100.0	14.3	14.7	> 17

* Mice one year of age at start.

Therefore, a few more S.G. tests were in the more severe grades ('2' or '3') with the diepoxides than with either of the two classifications of the monoepoxides. While none of the monoepoxides have been shown to be tumorigenic in the long-term tests, this S.G.T. probably indicates, as did the L.T.T., that the diepoxides are more severe in action.

It is obvious that, in our present state of knowledge of the relationship between these two tests, S.G.T. cannot be used to predict the results of the L.T.T. Probably, if the sebaceous glands are suppressed, one could assume, until a L.T.T. is completed, that the material is a potential tumorigen. This will not always be true; false positives have occurred. Contrariwise, if the S.G.T. is negative, it is unsafe to predict the results of L.T.T. In the cases discussed previously, a negative S.G.T. was usually associated with negative L.T.T. But, if this correlation is not perfect, grave consequences could result. Therefore, the S.G.T., in the opinion of the authors and based on these data, is merely a crude screen to indicate potential L.T.T. tumorigens; not a test to be used to form definite positive or negative conclusions concerning tumorigenesis.

Summary

Carcinogenic and acute toxicity potential were assayed while more than 60 epoxy compounds were being studied for commercial utility. Range-finding toxicity data are presented for 60, lifetime mouse skin-painting results are given for 28, and mouse sebaceous gland suppression results are listed for 26 compounds.

The epoxides studied ranged from extremely toxic to very low order of acute toxicity by peroral and inhalation routes in rats, and by the skin penetration route in rabbits. Skin irritation was generally mild, but several epoxides proved to be sensitizers in guinea pigs. Relationships discernible between molecular configuration and toxicity or irritation were tenuous at best.

None of eleven monoepoxides produced tumors during lifetime skin painting of mice, while five of 17 diepoxides were tumorigenic (butadiene dioxide, vinylcyclohexene dioxide, bis-(2,3-epoxy-2-methylpropyl) ether, a methylcyclohexylmethyl ether, and a modified bis phenol diglycidyl ether). The latter adduct was tumorigenic while both of its components were inactive when tested alone.

The median latent period for tumor production was 15 to 23 months, compared to three to five months for tumors produced by methyl cholanthrene, used as a positive control. Attempts, with one of these epoxides, to reduce the latent period by the use of dodecane as an accelerator, or by initiating skin-painting when mice were one year of age were not successful.

Some relationship was observed between the suppression of sebaceous glands after a two-week mouse skin-painting test, and tumor incidence in lifetime painting, but correlation was not sufficiently consistent to allow the brief test to be reliable for the prediction of carcinogenic potential.

No generalities about the toxic, irritative and carcinogenic hazards of handling epoxy compounds are justified by the presently known facts. Each compound must be individually studied to determine toxicity and carcinogenicity potential.

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WORLD HEAlth Org. tech. Report. Ser. no. 373

6.2 Fatty emulsifiers

The Committee found it convenient to consider the acetic acid and fatty acid esters of glycerol, and the corresponding additives with citric acid, lactic acid, tartaric acid and mixed tartaric and acetic acids in place of acetic acid, as a group of related compounds because of their complete hydrolysis during manufacture and/or digestion into acceptable constituents of the diet. Bearing in mind the established principle that no food additive should be used at a higher level than that needed to achieve the technological effect required, the Committee agreed on an acceptable daily intake for the whole group in terms of total fatty acids esters of glycerol, with the following proviso: any contribution made by liberated lactic or tartaric acid to the total dietary intake of these two acids must not exceed their respective acceptable daily intakes, set forth in the eighth and ninth reports of this Committee. No such limitation need be imposed in the case of citric acid because of the revised evaluation mentioned in a later section in this report. A new member, mixed acetic and tartaric and fatty acid esters of glycerol, was considered and included in the group evaluation. In the interest of simplicity the Committee renamed the individual members of this group as follows: acetic (or citric, lactic, tartaric, etc.) acid and fatty acid esters of glycerol.

Evaluation of diacetyltartaric acid and fatty acid esters of glycerol had been postponed at the last meeting because of the absence of suitable specifications. These have now been provided and an acceptable daily intake agreed which is, however, independent of that established for the group of glyceride esters mentioned above, the reason being that diacetyltartaric acid is not found naturally in the diet. The evaluation of sulfoacetic acid and fatty acids esters of glycerol and their sodium salts was postponed because of insufficient biological data. Moreover, the hydrolysis of these esters gives rise to components that are not acceptable dietary constituents, and this necessitates separate evaluation.

The group of polyglycerol esters of fatty acids was evaluated on the basis of extensive toxicological data provided for a typical member and a specification was prepared. Because of the similarity in their biochemical behaviour, all compounds complying with the agreed specification are covered by the evaluation, but related compounds, not complying with the specification, will require individual biochemical studies to exclude unforeseen toxicological effects.

The sucrose esters and sucroglycerides could not be evaluated because of inadequacy of available biochemical information and lack of proper

toxicological data on dimethyl formamide present as a contaminant in these compounds; such data might have enabled the Committee to set a limit for this unavoidable contaminant.

6.3 Natural stabilizers

Furcellaran and its salts could not be evaluated because of lack of relevant toxicological information but a tentative specification was prepared and is available. Carrageen and its salts are at present being used as food additives and for pharmaceutical purposes. The available toxicological data are, however, inadequate for the establishment of an acceptable daily intake. The Committee recommends that suitable information, particularly from the use of carrageen in man, should be provided within the next four years to allow a decision to be taken on permission to continue the use of this substance.

Notwithstanding the fact that gum arabic, karaya, tragacanth, carob bean gum and oat gum have been employed in and as food over many years in various countries, the Committee felt that the available toxicological information on these substances was insufficient for the establishment of acceptable daily intakes. However, tentative specifications have been prepared for all these substances except oat gum, and it was considered desirable that further studies with emphasis on absorption and fate in the human body should be carried out.

6.4 Steroid emulsifiers

Specifications could be established only for cholic and deoxycholic acids and their salts. These bile acids are physiological substances and were found to be limited in their use because of their bitter taste. An acceptable daily intake was established on the basis that a 5% variation in the daily output of bile salts is of normal physiological occurrence, so that an intake of corresponding magnitude would not contribute significantly to the normal body load.

6.5 Miscellaneous emulsifiers

No evaluation was possible for hydroxylated lecithin on the basis of the information available to the Committee. It was noted that bleached lecithin was included in the specification and evaluation given for lecithin in the seventh report of the Joint FAO/WHO Expert Committee on Food Additives.[1]

[1] *FAO Nutrition Meetings Report Series*, 1964, No. 35; *Wld Hlth Org. techn. Rep. Ser.*, 1964, **281**.

Annex 1

ACCEPTABLE DAILY INTAKES FOR MAN OF SOME EMULSIFIERS AND STABILIZERS

Compounds considered	Specifications available	Overall daily intake zone [a] (mg/kg body-weight)	
		Unconditional	Conditional
Sodium carboxymethylcellulose	Yes	0-30 [b]	Higher levels for dietetic or calorie control purposes
Hydroxypropylmethylcellulose	Yes		
Methylcellulose	Yes		
Methylethylcellulose	Yes		
Acetic acid and fatty acid esters of glycerol	Yes	0-100 [c]	
Citric acid and fatty acid esters of glycerol	Yes		
Lactic acid and fatty acid esters of glycerol [d]	Yes		
Mixed tartaric and acetic and fatty acid esters of glycerol [e]	Yes		
Diacetyltartaric acid and fatty acid esters of glycerol	Yes	0-25	25-50
Polyglycerol esters of fatty acids	Yes	0-12.5	12.5-25
Propylene glycol esters of fatty acids	Yes	0-20 [f] [c]	20-60 [f]
Cholic and deoxycholic acids and their salts	Yes	0-1.25	

[a] The first part of the overall acceptable daily intake zone is termed unconditional and this represents levels that can be safely employed without further expert advice. The second part of the zone is termed conditional and represents levels that can be employed safely but at which it is thought desirable that some degree of expert supervision and advice should be readily available.

[b] As the sum of these cellulose derivatives.

[c] As the sum of these fatty emulsifiers.

[d] The conditional zone of acceptability for the total intake of D(—)-lactic acid is 0-100 mg/kg.

[e] The zone of acceptability for the total food additive intake of tartaric acid is 0-6 mg/kg (unconditional) and 6-20 mg/kg (conditional).

[f] As propylene glycol.

Soap Sanit. Chem. 25 (12): 40-43, 147, 1949 172

TALL OIL

By C. B. F. Young,
George H. Eick
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TALL OIL is a by-product of the Kraft industry. In reality it is the sap of the pine tree. Appreciable amou... of this material can be recovered ..om the Southern Kraft mills which use long leaf, short leaf, slash, and loblolly pine as their raw material. Fair amounts are recovered in the paper mills located in the Wisconsin-Minnesota-Michigan area. Certain of the Canadian plants in the eastern part of the country also produce limited amounts of tall oil. Since tall oil is a by-product of the Kraft industry, the production of this interesting mixture of fatty and rosin acids depends upon the production of pulp.

The steps involved in the production of pulp are as follows: The logs in the woods are cut in sections of about five feet in length and from three to eight inches in diameter. They are brought to the mill and here debarked by being placed inside a large rotating drum. The debarked log is then fed into a chipper which is a round unit having at least four blades which cut the log into chips approximately two inches square and one-fourth inch thick. These are fed into digesters which contain organic and sodium sulfides, and the temperature is raised to 150° C. at 150 pounds pressure from three to four hours. At the end of this time the lignin or cementing material of the wood and sap is dissolved and put in a solution. The cellulose remains behind. The solution is drained off and washed free from the cellulose which is then formed into sheets of pulp.

In order to make the process economical the free alkali must be recovered from the solution. Pentuple effect evaporators are used to remove the water from the solids. As the per cent of solids increases, because of the removal of water from the whole, a salting-out effect occurs. It will be recalled that the lignin, sodium salts and the sap of the pine tree are all present in the solution. The sodium salts of the sap are sodium resinate, sodium oleates, sodium linoleates, etc. These materials, as can be readily seen, are ordinary soaps. As pointed out above, as the salt content of the solution increases, it being more soluble than the soap causes the latter to separate and float to the top of the solution. This is very detrimental to the paper mills as the floating soap gets on the tubes of the evaporator and prevents heat transfer which in turn prevents evaporation of the water. Thus the production of the plant is cut down. Most mills are now taking the liquid between the second and third evaporators and allowing this to remain in a holding tank for approximately thirty minutes at which time the soap, sometimes called black liquor sulfate soap skimmings or floating soap, rises to the top of the solution, is skimmed off and sent to holding tanks.

To follow through with the evaporation, the liquid is returned to the third effect evaporator and continued through the last one. Then this is fed into a furnace where the lignin and all combustible materials are burned off and the remaining water removed. Sodium sulfate or salt cake is added to make up for the loss and the resulting product is then put back in the solution and used to dissolve more wood chips.

The black liquor sulfate soap skimmings recovered between the second and third evaporator are allowed to settle in a holding tank for twenty-four to forty-eight hours and then any black liquor which settles is sent back to the paper manufacturing unit. The floating soap is put into tank cars and sent to us. We buy the skimmings from ten to twelve plants located from Texas to Virginia, blend the various soap stocks, and treat them with mineral acids to liberate the free fatty and rosin acids. By blending the soap stocks, a uniform product is produced for the customer during the four seasons of the year.

For many years the pine tree was considered to contain no fatty acids. None was found upon "tapping" the trees to obtain turpentine. However, the tree is not deeply cut for

* Presented before the 35th mid-year meeting of the National Association of Insecticide & Disinfectant Manufacturers, at the Hotel Drake, Chicago, June 13, 1949.

"tapping" and the fatty acids are found inside the tree. For this reason, the fatty acid portion was not discovered until the composition of the "black liquors" was determined.

Generally, tall oil consists of 50-52 per cent fatty acids, 41-43 per cent rosin acids, and seven per cent sterols or higher alcohols. A typical analysis of the fatty acid portion indicates: 48 per cent linoleic acid, 45 per cent oleic acid, and seven per cent saturated acids.(1) Oleic acid is an unsaturated acid found in animal and vegetable oils. Linoleic acid occurs also in vegetable oils. The rosin acids resemble abietic acid, which is found in various percentages in ordinary rosin. The unsaponifiables contain abundant quantities of sterols of which phytosterol is one of the primary constituents.

Small amounts of highly-colored and highly-odorous materials are also present in the oil. These materials have produced serious problems and sometimes barred the use of tall oil in many applications. In the last few years the number of uses for tall oil has greatly increased, and much research has been carried out to improve the color and odor of the oil. As a result of this work, refined tall oils are now available with greatly improved color and odor characteristics.

The Swedes were very active in first developing tall oil. Most of the refining processes were developed in Europe. Tall oil has been produced in the Scandinavian countries since about 1900, but was not produced in the United States until about 1930 (2). At that time it ranked as a fatty acid. At first it was offered as a substitute for commercial red oil, but as its properties became better known, new uses were found for it.

Tall is Swedish for pine. In Sweden, where the material was first developed, tall oil was known as *Tall-olja* which means oil of the pine. The Germans called it *Tallol;* but, inasmuch as we already had a product in America known as pine oil, it was given the name "tall oil" upon the suggestion of the War Production Board.

Tall oil had a very limited market in this country prior to World War II. There was a reluctance on the part of potential users to attempt to adapt crude and refined tall oils to their formulas. Many of the difficulties encountered in trying to use the oil would not have occurred had the processor realized that he was attempting to substitute a material, which varies over wide limits, for materials with which he was familiar.

Tall Oil in Soap

TALL OIL has proved to be a useful raw material to the soap maker. The high percentage of unsaturated groups in tall oil and its low titre have a tendency to increase the affinity toward mineral oil as well as aliphatic and aromatic compounds. It is a well known fact that rosin acids in soap increase its affinity toward mineral oil, besides the sterols or unsaponifiables. By making use of these rosin acids in soap, low water solubility results as indicated by a lack of suds unless water softeners are added, such as: "Nullapon B," "Sequestrene A," tetrasodium pyrophosphate, silicates, or equivalents. The addition of these agents will lessen the forces existing among the various surface particles of water, or technically, lower the interfacial or surface tension. Dilute tall oil soap solutions have abnormally low surface tension; because of this fact, tall oil soaps have the remarkable ability to form a colloidal suspension. This suspension adsorbs and entangles the dirt particles and enables them to be rinsed away. Grease, oil and dyestuffs are emulsified and washed away as small droplets surrounded by portions of the soap solutions, which serve as a protective colloid. For this reason, tall oil soaps are widely used for the degreasing of aeroplanes, automotive engines, in the cleaning of asphalt tile and linoleum, as well as in the preparation of metal stock prior to plating. Incidentally, in industrial cleaning, sudsing is not essential.

It can be shown that in a film on the horizontal surface of pure water, the sodium parts of the molecules, including the carboxyl group to which the sodium is attached, lie in the water while the other ends of the molecules (hydrocarbon part) are out of the water. A fat or grease which is insoluble in pure water is soluble in the hydrocarbon part of the soap molecule. The soap molecule then acts as a connecting link, as one part of the soap dissolves the fat and the other part dissolves in the water. The globules of soap molecules in water have the sodium ends of the molecules pointing outward and in contact with the water, while the hydrocarbon parts of the molecules are directed inward and in contact with each other. (For other information on surface activity refer to Item 10 of the Reference.)

The cleaning properties of tall oil soaps are excellent and they are used for: cleaning overalls and wiping rags in industrial laundries; wool scouring in the textile industry; in brown bar laundry soaps; in spray dried powdered or bead soaps; in scrub, paste, and liquid hand soaps, as well as in the cleaning of metal stock, such as: zinc, aluminum, tin, brass, copper and steel prior to a plating operation. Other uses of tall oil soaps include; emulsifiable disinfectants, coal tar disinfectants, amine emulsions, sulfonated oils, insecticides, plant sprays, and cattle dips.

Tall oil soaps have good sudsing properties in soft water, but this property decreases as the hardness of the water increases. The drop in sudsing ability of tall oil soaps is faster than that of stearate soaps, but this drop can be overcome by blending other fats with the oil to make soap. Upon saponification, for example, of a mixture of 35-40 per cent of high titre fats with 65-60 per cent of tall oil, the soap produced will have sudsing properties comparable to high titre fats. Because of its comparatively low alkali requirement, tall oil is preferred in the preparation of soaps using an expensive alkali, such as triethanolamine.(2)

As the degree of hydrogenation increases, the hardness of the soap increases, and the amount of salt required for salting out decreases. Lathering quality is inversely proportional to the hardness of the acids; and because of this property, the use of hydrogenated tall oil has some limitations. (3)

The emulsifying and foaming powers of soaps made from purified or refined tall oil are always better than those of soaps precipitated from crude tall oil. The foaming power of soaps made from purified tall oil is as high as that of pure fat soaps; and the emulsifying power is satisfactory. A soap which has a higher emulsifying power than 100 per cent fat soaps, may be prepared from a mixture of tall oil and hydrogenated fats. Although soaps from crude oil have too low an emulsifying power, liquid soaps may be prepared exclusively from refined tall oil. (4)

Low surface tension of tall oil soap solutions creates a remarkable ability to form colloidal suspensions . . . making these soaps superior for degreasing metals, cleaning linoleum, tile, etc.

Tall Oil in Other Soaps

IN PREPARING a brown laundry soap, the full boiled process is generally employed where the soapmaker intends to recover the glycerine; in this case, the tall oil should be saponified separately and added to the tallow which is then saponified. By doing this, the soap yield will be increased; otherwise, a large percentage of the fatty acids from the tall oil are lost in the "nigre." A full boiled soap is run into a crutcher and up to 50 per cent "Facoil CB" (Fatty Acid Oil)* added. The oil is saponified at 180° F. for about an hour longer than is ordinarily required in order to break down the rosin acids in the tall oil from polymers to monomers. This eliminates one of the causes for stickiness and a probable source for complaint. Then fillers are added to act as buffering agents to attain the proper pH and neutralize any soil acidity. After agitation the soap is run into frames at 149-158° F.

Tall oil may be used with whole oils, such as: corn, linseed, soya or vegetable fatty acid foots, in the manufacture of scrub or paste soaps. Although 100 per cent tall oil is being used successfully by some soapers in scrub and liquid formulae, we usually recommend 50 per cent tall oil in conjunction with any other oil, fatty acid or mixture thereof. The kettle is charged with the alkali and water and then heated to 180° F. About one per cent of a polyaminocarboxylic acid is added in the liquid form before the fatty acids are admixed at a rate not exceeding one gallon a minute. If desired, one-third of the pine oil is placed in the kettle to act as an emulsifying agent. If a firmer soap is desired, 10-15 per cent of the potash can be replaced with caustic soda.

A typical formula for a 40 per cent liquid hand soap is as follows:

A.

200 lbs.	*Facoil CB
200 lbs.	Potash at 33° Baumé
230 lbs.	soya bean oil

B. (30 per cent Potash Soap)

190 lbs.	*Facoil CB
190 lbs.	Linseed Oil
10 lbs.	Pine Oil
92 lbs.	Potash
600 lbs.	Hot Water

C. (15 per cent Potash Soap)

5 parts	*Facoil CB
10 parts	Coconut Oil F.A.
9 parts	50° Bé Potash
1 part	Potassium Chloride
75 parts	Distilled Water

* Facoil, trade mark of National Southern Products Corp., New York, refined tall oils.

Mix the oils and potash at 80° C. In the above illustration, the linseed oil permits smoother boiling. Pine oil in hand and disinfectant soaps acts as a masking agent; it exerts a solvent effect thereby aiding in the removal of grease.

The following formulae are suggested for scrub soaps:

		Parts
A.	Tall Oil Potash Soap	30
	Sodium Carbonate	4
	Pine Oil	7
	Trisodium Phosphate	4
	Water	55
B.	*Facoil CB (Sap. No. 174)	20
	85% Caustic Potash	4
	Trisodium Phosphate	4
	Pine Oil	20
	Water	52

Tall oil can be used for textile scouring to replace all or part of the red oil content. In this application, sodium carboxymethyl cellulose tends to increase the colloidal characteristics of the fatty acid soaps. Usually, they are prepared by the semi-boiled process.

		parts
A.	Palm Kernel Oil or Coconut	60
	*Facoil CB	40
	Lye at 36° Baumé to saponify	

One or two pounds of tall oil are added for each gallon capacity of the tank to be used to prepare a soap preferred by many textile mills. When the scouring operation requires the use of free alkali or soap builders, such as soda ash, they should be used with the tall oil soap. The amount of soap builders or excess alkali should be about the same as that required for other unbuilt soaps. Tall oil soap is readily removed from the scoured textile material by rinsing and no residual odor remains after drying, but it reacts with the calcium and magnesium salts in hard water to form insoluble soaps that cause decoloration and staining of textile fabrics. Therefore, when tall oil soap is used, water softening equipment should be checked frequently to assure that the water used is of zero hardness.

It is possible to replace the entire soap requirements of most textile mills with tall oil soap. The best method for this replacement is to carry out a trial operation on as large a scale as possible with replacement with tall oil in 5-10 per cent steps. (5)

Tall Oil in Insecticides

MANY companies who manufacture agricultural and insecticide sprays have made wide use of tall oil as the insecticide sticker. In certain types of sprays tall oil is used as an adhesive. Generally, it is used "as is" in these formulations. In some cases, it might be desirable to saponify a portion of it with caustic soda leaving a portion of the tall oil in an "as is" state for the adhesive. Thus, a combined emulsifying and adhesive agent is produced. Insects and other plant parasites are combatted with a solution of tall oil to which may be added tobacco extract, nicotine or petroleum solvents and a suitable stabilizer.

Some of the base materials used in the field of disinfectants are the sodium, potassium, ammonium and complex salts of tall oil. Disinfectants are produced by incorporating into soap solutions cresol and materials which have a high phenol coefficient. Using tall oil soaps as the emulsifying agent, general cleaners having disinfectant properties are made from tar acid oils. A stable or semi-stable emulsion may be produced by diluting the stock solution with water.

To prepare a disinfectant, an emulsion is made of tar acids or tar acid oils with "liquid sodium resinate" (tall oil soap) obtained from sulfate black liquor. A mixture of about 70-75 per cent by weight of tar acid oil (25 per cent tar acids) and 30-25 per cent by weight of liquid resinate is heated to about 75° C., then agitated until the mixture appears to be homogeneous. Enough resinate should be used to give a pH of about eight.

The preceding formula and the two that follow were prepared by Hyde. (6)

Formula No. I

	% by weight
Tar acid oil (25% tar acids)	68
Liquid resinate	30
Castor oil soap	2

Formula No. II

	% by weight
Insecticide oil	56.0
High boiling tar acids	4.0
Water gas tar distillate	8.0
Liquid resinate	30.0
Castor oil soap	2.0

The addition of water to the two formulas produces a stable emulsion type disinfectant.

The metallic soaps of tall oil are used for fungicidal applications. They are easily decomposed by the acids liberated during fungus growth processes and liberate metal ions. Another property that contributes to their applications as fungicides is their water insolubility. The two most frequently used are the copper and zinc soaps, and the copper has been found to be about five times as effective as the zinc soap. The soaps may be used to treat cloth, rope, concrete, and wood. The copper soap has been used to mildewproof cloth. Specification T-1452, Amendment No. 6 of the Corps of Engineers of the U. S. Army "Processing Sandbag Fabrics for Mildewproofing" permits tall oil to be used for extending the naphthenic acids used in mildewproofing compounds. (5)

Although most of the water-insoluble soaps find use in the paint and varnish fields as driers, the copper soaps have fungicidal properties. They are soluble in petroleum thinners and are therefore especially suitable for use in paints for coating ship bottoms.

To prepare the copper soap, the tall oil is boiled with aqueous caustic soda or potash to form the soap solution. The soap solution is then diluted to about 20 per cent solids, the temperature maintained at about 200°F. and the required amount of metal added. The metal soap is formed by double decomposition and precipitates to the bottom of the vessel where it is separated, washed with boiling water and heated to 350°F. to remove water. Copper soap has also been prepared by fusing the oxide or hydroxide of the metal directly with the oil. (7)

Liquid degreasing agents to be used for degreasing aeroplanes, automobile engines, oil tankers, the surfaces of metals that become greasy during manufacturing operations, or metal parts prior to the electro-plating operation, have been formulated using tall oil.

Saponification with caustic potash gives a jel-like soap and as much as 50 per cent of the potash may be replaced with sodium hydroxide. A sufficient amount of alkali should be added to completely saponify all the oil and to have about three per cent free alkali in the finished product. To prevent soap particles from settling out, about 15-30 per cent of cresylic acid may be added. (8)

Formula A

To remove slushing and shearing compounds and rust preventatives:

	%
Facate*	5
Sodium Orthosilicate	95

This material acts as a lifter in removing dirt, and is more efficacious than mineral salts or rosin compounds.

Formula B

	%
Facate*	5
Sodium Orthosilicate	50
Tri Sodium Phosphate	45

Any alkali may be used as an alternate in the above formulations, such as ortho, meta, or sesqui silicate.

Formula C

For cleaning Aluminum, Brass, Tin, etc.

Facate*	1 part by weight
Naphtha or mineral spirits	2 parts by weight

Add above to 10—90 per cent water

Formula D

Automotive engines (will not attack heavy metal):

	%
Facate*	15
Sodium Metasilicate	80
Soda Ash	5

Used at 4-6 oz./gallon and 180-212° F. Rinse.

* Facate is a trade name product of National Southern Products Corp. made from "Facoil CS" or CB by saponifying with caustic soda or other alkali.

Liquid Tall Oil Soap

IN some cases, when liquid tall oil soaps were prepared, manufacturers witnessed a cloudy or hazy appearance after prolonged standing. In order to extend the shelf life by making these products eye-appealing, our company has investigated this problem with a view to finding the direct cause of this situation. We soon discovered many variables. The sterols (higher alcohols, as phytosterol) or as they are commonly called, unsaponifiables, had a tendency to cause a flocculent precipitate; but, these were easily eliminated by refrigerating and filtering. Another variable appeared to be iron picked up while the tank car was in transit from the refinery to the customer's soap plant. This was detected as a reddish-brown ring around the lip of the bottle. By adding a very small percentage of a reducing agent, as sodium hydrosulfite, the ferric hydroxide was easily reduced to ferrous hydroxide.

(*Turn to Page 147*)

Tall Oil

(From Page 43)

Potash relatively free of chloride was tried in the preparation of liquid soaps, and even when necessary precautions were taken, a slight haze was visible after prolonged standing. Various chemicals were then tried, for example: a commercial sodium silicate, tetrasodium pyrophosphate, tetrapotassium pyrophosphate, trisodium phosphate, an excess of sodium hexametaphosphate to dissolve the insoluble calcium salts which might occur from the glass itself or from the hardness of the water used.

Sequestering agents opened up a new avenue of possibilities to the soaper as a means to check metal trace effects, as well as a surface modifier. In fact, they showed certain distinct advantages over the inorganic polyphosphates because they are highly compatible with organic groups at various concentrations, and they are remarkably stable in a water solution at various temperatures over the hydroxyl ion concentration range.

These sequestrene products are polyaminocarboxylic acids and manufactured under various trade names, as: "Nullapon B," "Kalox," "Sequestrene A," and equivalents. Usually, a maximum of one per cent is all that is needed to eliminate any cloudiness which might occur in the prepared liquid soap.

Kranich's (9) full and interesting report states:

> The glass containers themselves contributed definitely to the breakdown in clarity of the products. This is explained by saying that once the sodium silicate derived from the glass hydrolyzes to silicic acid, it is precipitated as a flocculating gelatinous meta-silicic acid (ortho silicic acid is soluble), the dissolution of this precipitate being prevented perhaps by the adsorption on its surface of a film of calcium stearate soap. Moreover, the calcium salts of the lower fatty acid homologues, decidedly more soluble, are readily dispersed.

In conclusion, tall oil is the cheapest source of fatty acids in the world. Because of this, it has proven to be a very useful raw material to the soapmaker. Physically, it is different today than the oil marketed at the beginning of the war. Through a wide research and development program since the war's end, refined tall oils are now non-crystallizing, light in color, less odoriferous, and uniform in consistency from tank car to tank car. Present research calls for the elimination of all of the sulfur compounds, and removal of the color bodies to make it more stable and attractive to the insecticide and disinfectant industry.

It takes a higher temperature and longer time to completely saponify the rosin acids in tall oil after the fatty acids have been saponified. This precaution eliminates stickiness, a frequent complaint we encounter whenever tall oil soap products are prepared for the first time.

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TOXICOLOGICAL DATA

BULLETIN T-114

PALE ROSIN
(Gum, Wood, and Tall Oil)

SUMMARY OF TOXICOLOGICAL INVESTIGATIONS

Chemical Composition

"Rosin" is the common name applied to a complex mixture of mutually soluble organic compounds found in various species of the pine tree.

Commercial rosins are obtained from three important sources:

1. Gum obtained by tapping living pine trees (gum rosin).

2. Crude resin extracted by solvent from aged pine stump wood (wood rosin).

3. Waste liquors recovered from wood-pulping operations (tall oil rosin).

The gum, wood, and tall oil rosins produced from these sources contain acidic and neutral fractions, the proportions of which vary with the source and degree of refining. Refining reduces the color of rosin from a dark brown to very pale yellows, with the intensity of residual color dependent on grade.

The composition of pale rosins varies, depending on the source. Pale gum and wood rosins are composed of approximately 90 percent resin acids and 10 percent neutral materials. Pale tall oil rosin may contain from 2 to 10 percent fatty acids (mainly C_{18}), 5 to 10 percent neutral materials, and up to 90 percent resin acids.

The resin acid fraction in all three types is approximately the same, consisting of nine basic diterpenoid monocarboxylic derivatives of alkylated hydrophenanthrenes. These are generally divided into two groups: the

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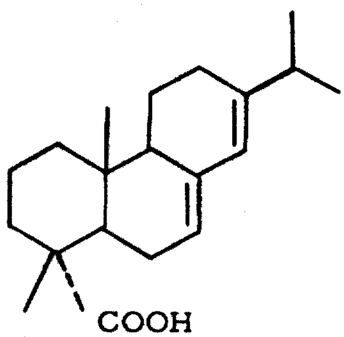
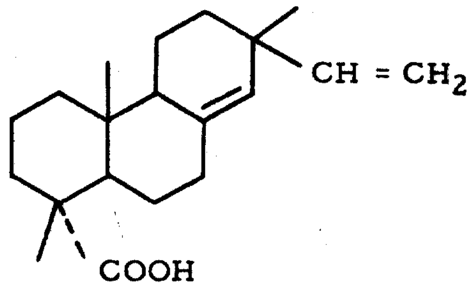
abietic type and the pimaric type. They occur in a ratio of about 3 abietic to 1 pimaric. The structure of a typical resin acid of each group is shown below.



Abietic Acid



Pimaric Acid

The neutral fractions of all three types of rosin are similar. About half consists of esters of resin acids, and the remainder a mixture of sterols, resenes, terpene alcohols, terpenes, and other hydrocarbons.

Physical Properties

Pale rosins are clear resinous solids that range in color from dark amber to very pale yellow. They are available commercially in several color grades. Their softening points* range from 80 to 90°C, and their acid numbers from 150 to 170, depending on their source and neutral fraction content.

Rosin is essentially insoluble in water, but soluble in organic solvents, and in fats and oils. Pale unmodified rosins will absorb oxygen on exposure to air, the rate depending on temperature and on whether the rosin is crushed or solid.

The samples used for the toxicological investigations reported below were composite blends of a representative cross section of pale rosin samples from various production facilities and locations in the United States.

Acute Toxicity

Pale gum, wood, and tall oil rosin, administered as a 30 percent solution in corn oil, gave the following LD_{50} values for rats, mice, and guinea pigs.

*Hercules drop method

	LD50 (mg./kg.)		
	rats	mice	guinea pigs
pale gum rosin	7600	4600	4100
pale wood rosin	8400	4100	4100
pale tall oil rosin	7600	4600	4600

Subacute Oral Toxicity

Pale gum, wood, and tall oil rosins have been fed to young albino rats for 90 days at dietary levels of 5, 1, 0.2, 0.05, and 0.01 percent. Each was incorporated into ground laboratory diets as 30 percent corn oil solutions. Observations during the course of feeding included: general appearance, growth, food intake, hematology, urinalysis. After 90 days, surviving animals were sacrificed, and pathologic studies conducted. Autopsies were performed and organ weights obtained for liver, kidney, spleen, gonad, heart, and brain. Microscopic examination included: brain, liver, spleen, stomach, small intestine, colon, pancreas, kidneys, urinary bladder, adrenals, gonads, thyroid, parathyroid, lymph nodes, heart, lungs, bone marrow, muscle, prostate, and uterus.

At the 5 percent dietary level, marked food refusal occurred with all three types of rosin and the rats lost weight rapidly during the first few days. All rats died within two weeks.

At the 1 percent dietary level, weight gain was depressed during the first two weeks of the study, along with food consumption. After two weeks, food consumption and weight gain were comparable to controls. No mortality occurred, and urinalysis and hematology were normal throughout the 90 days. At autopsy, the animals showed a higher liver weight than control animals. Other organ weights were comparable to controls. Histologic examination revealed no significant alterations in any organs, including the liver.

At the 0.2, 0.05, and 0.01 percent dietary levels, no significant differences from control animals were detected with any of the three pale rosins.

Chronic Oral Toxicity

Pale gum, wood, and tall oil rosins have been fed for two years to young albino rats and young beagle dogs at dietary levels of 1 and 0.05 percent. In addition, pale wood and tall oil rosin have been fed for the same period to rats at a dietary level of 0.2 percent. All three types of rosin were added to the diet as 30 percent solutions in corn oil. All diets, including controls, were adjusted to the same level of corn oil. Observations during the two years included gross symptoms, mortality, food intake, body weight, hematology, urinalysis, liver and kidney function tests, and tumor incidence. Autopsies after two years of feeding included individual organ weights for livers, kidneys, spleens, gonads, brain, hearts, thyroids, and adrenals. Histologic studies included all of the tissues described above in the subacute study for rats and dogs. In addition, the aorta, gall bladder, peripheral nerves, and spinal cords of the dogs were examined.

At the 1 percent dietary level, food consumption of rats was approximately 10 percent below control level with all three types of pale rosin. As a result, the growth rate was slightly depressed. No significant differences in food consumption and growth rate were observed with the dogs. Hematology, urinalysis, and liver and kidney function tests were all within normal limits throughout the study. Analysis of organ weight data revealed enlargement of the liver in both species of animals with all three pale rosins. The liver enlargement was less pronounced than that observed after 90 days of feeding. All other organ weights were comparable to controls. There were no histologic findings in any of the organs and tissues, including the liver, of the rats or dogs which could be attributed to the ingestion of any of the pale rosins. Tumor incidence with all three rosins was equal to, or less than, the incidence in the control animals.

At the 0.2 and 0.05 percent dietary levels, no significant differences from control animals were detected with any of the three pale rosins, either in rats or in dogs.

FDA Status

For uses where it may come in contact with food, Pale Rosin has FDA clearance as specified in Section 121.2592 of the Code of Federal Regulations (Federal Register, 12/23/64 p. 18216), and in 14 other FDA regulations. Consult a Hercules representative for reference to regulations specific to finished forms of substances or articles that come in contact with food.